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(54) Title: HUMAN MONOCLONAL ANTIBODY

(57) Abstract

This invention relates to novel human monoclonal antibodies (mAbs) and to the genes encoding same. More specifically, this invention relates to human monoclonal antibodies specifically reactive with an epitope of the fusion (F) protein of Respiratory Syncytial Virus (RSV). Such antibodies are useful for the therapeutic and/or prophylactic treatment of RSV infection in human patients, particularly infants and young children.

HUMAN MONOCLONAL ANTIBODY

Field of the Invention

5   This invention relates to novel human monoclonal
antibodies (mAbs) and to the genes encoding same. More
specifically, this invention relates to human monoclonal
antibodies specifically reactive with an epitope of the
fusion (F) protein of Respiratory Syncytial Virus (RSV).
10   Such antibodies are useful for the therapeutic and/or
prophylactic treatment of RSV infection in human
patients, particularly infants and young children.

Background of the Invention

15   Respiratory syncytial virus (RSV) is the major
cause of lower respiratory disease in children, giving
rise to predictable annual epidemics of bronchiolitis
and pneumonia in children worldwide. The virus is
highly contagious, and infections can occur at any age.
20   Comprehensive details concerning RSV infection and its
clinical features can be obtained from excellent recent
reviews by McIntosh, K. and R. M. Chanock, In:
"Respiratory Syncytial Virus", Ch. 38, B.N. Fields ed.,
Raven Press (1990) and Hall, C.B., In: "Textbook of
25   Pediatric Disease" Feigin and Cherry, eds., W.B.
Saunders, pgs 1247-1268 (1987).
          RSV is distributed worldwide. One of the most
remarkable features of the epidemiology of RSV virus, as
mentioned above, is the consistent pattern of infection
30   and disease. Other respiratory viruses cause epidemics
at irregular intervals or exhibit a mixed
endemic/epidemic pattern, but RSV is the only
respiratory viral pathogen that produces a sizable
epidemic every year in large urban centers. In the

temperate areas of the world, RSV epidemics have
occurred primarily in the late fall, winter or spring
but never during the summer. The occurrence and spread
of infection within a community is characteristic and
5   easily diagnosed, leading to sharp rises in cases of
bronchiolitis and pediatric pneumonia and the number of
hospital admissions of young children with acute lower
respiratory tract disease. Other respiratory viral
agents that occur in outbreaks are rarely present at the
10   same time as RSV.

Primary RSV infection occurs in the very young.
Zero to 2 year old infants are the most susceptible and
represent the primary affected population. In this
group, 1 out of 5 will develop lower respiratory (below
15   larynx) disease upon infection and this ratio stays the
same upon reinfection. By 1 year of age, 25-50% of
infants have specific antibodies as a result of natural
infection and this is close to 100% by age 4-5. Thus,
virtually all children have been infected before they
20   have entered school.

Age, sex, socioeconomic and environmental factors
can all influence the severity of disease.
Hospitalization is required in 1-3% of cases of RSV
infection and is usually of long duration (up to 3
25   weeks). The high morbidity of RSV infection, especially
in infancy, has also been implicated in the development
of respiratory problems later in life. With current
intensive care in the U.S. and the other developed
countries, overall mortality for normal subjects is low
30   (less than 2% of hospitalized subjects). However,
mortality is much higher in less developed countries
and, even in developed countries, mortality is high in
certain risk groups such as in infants with underlying
cardiac condition (cyanotic congenital heart disease) or

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respiratory disease (bronchopulmonary dysplasia) where
the progression of symptoms may be rapid. For instance,
mortality in infants with cyanotic congenital heart
disease has been reported to be as high as 37%. In
premature infants apneic spells due to RSV infection may
occur and, in rare cases, cause neurologic or systemic
damage. Severe lower respiratory tract illness
(bronchiolitis and pneumonia) is most common in patients
under six months of age. Infants who have apparently
recovered completely from this illness may display
symptomatic respiratory abnormalities for years
(recurrent wheezing, decreased pulmonary function,
recurrent cough, asthma, and bronchitis).

Immunity to RSV appears to be short-lived, thus
reinfections are frequent. The mechanisms by which the
immune system protects against RSV infection and
reinfection are not well understood. It is clear,
however, that immunity is only partially protective
since reinfection is common at all ages, and sometimes
occurs in infants only weeks after recovery from a
primary infection. Both serum and secretory antibodies
(IgA) have been detected in response to RSV infection in
adults as well as in very young infants. However, the
titers of serum antibodies to the viral F or G
glycoprotein, as well as of neutralizing antibodies
found in infants (1-8 months of age) are 15-25% of those
found in older subjects. These reduced titers may
contribute to the increased incidence of serious
infection in younger children.

Evidence for the role of serum antibodies in
protection against RSV virus has emerged from
epidemiological as well as animal studies. In adults
exposed naturally to the virus, susceptibility
correlated well with low serum antibody level. In

infants, titers of maternally transmitted antibodies
correlate with resistance to serious disease [Glezen,
W.P. *et al.*, *J. Pediatr.* 98:708-715 (1981)]. Other
studies show that the incidence and severity of lower
5   respiratory tract involvement is diminished in the
presence of high serum antibody [McIntosh, K. *et al.*, *J. Infect. Dis.* 138:24-32 (1978)] and high titers of
passively administered serum neutralizing antibodies
have been shown to be protective in a cotton rat model
10   of RSV infection [Prince, G. A. *et al.*, *Virus Res.* 3:193-206 (1985)].

Children lacking cell-mediated immunity are unable
to overcome their infection and shed virus for many
months in contrast to children with normal immune
15   systems. Similarly, nude mice infected with RSV virus
persistently shed virus. These mice can be cured by
adoptive transfer of primed T cells [Cannon, M. J. *et al.*, *Immunology* 62:133-138 (1987)].

In summary, it appears that both cellular and
20   humoral immunity are involved in protection against
infection, reinfection and RSV disease and that although
antigenic variation is limited, protective immunity is
not complete even after multiple exposures.

RSV, belonging to the family paramyoxoviridae, is a
25   negative-strand unsegmented RNA virus with properties
similar to those of the paramyxoviruses. It has,
however been placed in a separate genus Pneumovirus,
based on morphologic differences and lack of
hemagglutinin and neuraminidase activities. RSV is
30   pleomorphic and ranges in size from 150-300 nm in
diameter. The virus matures by budding from the outer
membrane of a cell and virions appear as membrane-bound
particles with short, closely spaced projections or
"spikes". The RNA genome encodes 10 unique viral

polypeptides ranging in size from 9.5 kDa to 160 kDa
[Huang, Y. T. and G. W. Wertz, _J. Virol_. 43:150-157
(1982)]. Seven proteins (F, G, N, P, L, M, M2) are
present in RSV virions and at least three proteins (F,

5   G, and SH) are expressed on the surface of infected
cells. The F protein [SEQ ID NO: 20] has been
conclusively identified as the protein responsible for
cell fusion since specific antibodies to this protein
inhibit syncytia formation _in vitro_ and cells infected

10   with vaccinia virus expressing recombinant F protein
form syncytia in the absence of other RSV virus
proteins. In contrast, antibodies to the G protein do
not block syncytia formation but prevent attachment of
the virus to cells.

15   RSV can be divided into two antigenically distinct
subgroups, (A & B) [Mufson, M. A. _et al._, _J. Gen'l.
Virol_. 66:2111-2124 (1985)]. This antigenic dimorphism
is linked primarily to the surface attachment (G)
glycoprotein [Johnson, R. A. _et al._, _Proc. Nat'l. Acad._

20   _Sci. USA_ 84:5625-5629 (1987)]. Strains of both group A
and B circulate simultaneously, but the proportion of
each may vary unpredictably from year to year. An
effective therapy must therefore target both subgroups
of the virus and this is the reason for the selection of

25   the highly conserved surface fusion (F) protein as
target antigen for mAb therapy as will be discussed
later.
        The induction of neutralizing antibodies to RSV
virus appears to be limited to the F and G surface

30   glycoproteins. Of these two proteins, the F protein is
the major target for cross-reactive neutralizing
antibodies associated with protection against different
strains of RSV virus. In addition, experimental
vaccination of mice or cotton rats with F protein also

results in cross protection. The antigenic relatedness of the F protein across strains and subgroups of the virus is reflected in its high degree of homology at the amino acid level. In contrast, in the two subgroups and

5 various strains of RSV, antigenic dimorphism was linked primarily to the G glycoprotein. The F protein has a predicted molecular weight of 68-70 kDa; a signal peptide at its N-terminus; a membrane anchor domain at its C terminus; and is cleaved proteolytically in the

10 infected cell prior to virion assembly to yield disulfide linked F_2 and F_1. Five neutralizing epitopes have been identified within the F protein sequence [SEQ ID NO: 20] and map to residues 205-225; 259-278; 289-299; 483-488 and 417-438. Studies to determine the

15 frequency of sequence diversion in the F protein [SEQ ID NO: 20] showed that the majority of the neutralizing epitopes were conserved in all of the 23 strains of RSV virus isolated in Australia, Europe, and regions of the U.S. over a period of thirty years. In another study,

20 seroresponses of forty three infants and young children to primary infection with subgroup A or a subgroup B strain showed that responses to homologous and heterologous F antigens were not significantly different, while the G proteins of the subgroup A and B

25 strains were quite unrelated. Moreover, antibody inhibition of virus-mediated cell fusion *in vitro* versus inhibition of infection correlates best with protection in animal models and fusion inhibition is primarily restricted to F protein specific antibodies.

30 Prophylactic treatment for RSV infection is thus desirable for the high risk groups of children as well as for all children in underdeveloped countries. However, a vaccine for RSV infection is not currently available. Severe safety issues surrounding an

attenuated whole virus vaccine tested in the 1960s, as
well as the potential of induced immunopathology
associated with the newer candidate subunit vaccines
make the prospects of a vaccine in the near future
5   appear remote. To date one drug therapy, Ribavirin, a
broad spectrum antiviral, has been approved. Ribavirin
has gained only minimal acceptance owing to problems of
administration, mild toxicity and questionable efficacy.
In the majority of cases, hospitalized children receive
10   no drug therapy and receive only intensive supportive
care which is extremely costly. It is clear that there
is a need for a safe, effective and easily administered
drug for the treatment of RSV infection.
       The use of passive antibody therapy in humans is
15   well documented and is being used to treat other
infectious diseases such as hepatitis and
cytomegalovirus. The feasibility of passive antibody
treatment/protection against RSV has been well
established using animal models. Most of the earlier
20   passive transfer studies in animals against infectious
agents, including RSV, utilized murine mABs. Studies in
animals have clearly demonstrated that polyclonal and
monoclonal antibody against both F and G glycoprotein
can confer passive protection in RSV virus infection
25   when given prophylactically or therapeutically [Prince,
et al., supra]. In these studies, passive transfer of
neutralizing F or G mAbs to mice, cotton rats or
monkeys, significantly reduce or completely prevent
replication of the RSV virus in the lungs. However, as
30   discussed above, clearly, the F protein is the more
important target for antibody therapy.
       Recently, the FDA has approved for use intravenous
gammaglobulins (IVIG) isolated from pooled human sera.
Initial reports from this study had been encouraging

[Groothuis, J. R. *et al.*, <u>Antimicrob. Agents Chemo.</u> 35(7):1469-1473 (1991)]. However, generic shortcomings of IVIGs exist and include, without limitation, the fact that such products are human blood derived and grams of

5   antibody often need to be administered to achieve an effective dose.

Alternatively, monoclonal antibodies have been employed. The advantages of such an approach include: a higher concentration of specific antibody can be

10   achieved thereby reducing the amount of globulin required to be given; the reliance on direct blood products can be eliminated; the levels of antibody in the preparation can be more uniformly controlled and the routes of administration can be extended. While passive

15   immunotherapy employing monoclonal antibodies from a heterologous species (e.g., murine) has been suggested (See: PCT Application PCT/US94/08699, Publication No. WO 95/04081), one alternative to reduce the risk of an undesirable immune response on the part of the patient

20   directed against the foreign antibody is to employ "humanized" antibodies. These antibodies are substantially of human origin, with only the Complementarity Determining Regions (CDRs) being of non-human origin. Particularly useful examples of this

25   approach are disclosed in PCT Application PCT/GB91/01554, Publication No. WO 92/04381 and PCT Application PCT/GB93/00725, Publication No. WO93/20210. Clinical trials are on-going to evaluate the efficacy of humanized antibodies for treatment of RSV infection in

30   young children.

A second and more preferred approach is to employ fully human mAbs. Unfortunately, there have been few successes in producing human monoclonal antibodies through classic hybridoma technology. Indeed,

acceptable human fusion partners have not been identified and murine myeloma fusion partners do not work well with human cells, yielding unstable and low producing hybridoma lines. However, recent advances in
5 molecular biology and immunology make it now possible to isolate human mABs, particularly directed against foreign infectious agents.

Fully human mAbs to RSV F protein [SEQ ID NO: 20] remain a desirable option for the treatment of this
10 disease. Although some success has been reported in obtaining fragments of such mAbs [Barbas, C.F. *et al.*, Proc. Nat'l. Acad. Sci. USA 89:10164-10168 (1992); Crowe, J. E. *et al.*, Proc. Nat'l. Acad. Sci. USA 91: 1386-1390 (1994) and PCT application number
15 PCT/US93/08786, published as WO94/06448, March 31, 1994)], the achievement of such results is not straightforward. Novel human mABs, when and however obtained, are particularly useful alone or in combination with existing molecules to form
20 immunotherapeutic compositions.

There exists a need in the art for useful prophylactic compositions for the prevention or passive treatment of RSV.

25 **Brief Description of the Invention**

In one aspect, this invention provides fully human monoclonal antibodies and functional fragments thereof specifically reactive with an F protein epitope of RSV and capable of neutralizing RSV infection. These human
30 mABs specific for the F protein of RSV virus may be useful to passively treat or prevent infection.

In another aspect, the present invention provides modifications to neutralizing single chain Fv fragments (scFV) specific for the F protein of RSV produced by

random combinatorial cloning of human antibody sequences
and isolated from a filamentous phage Fab display
library.

In still another aspect, there is provided a
5   reshaped or altered human antibody containing human
heavy and light chain constant regions from a first
human donor and heavy and light chain variable regions
or the CDRs thereof derived from human neutralizing
monoclonal antibodies for the F protein of RSV derived
10   from a second human donor.

In yet another aspect, the present invention
provides a pharmaceutical composition which contains one
(or more) altered or reshaped antibodies and a
pharmaceutically acceptable carrier.

15   In yet another aspect, the invention provides a
pharmaceutical composition comprising at least one dose
of an immunotherapeutically effective amount of the
reshaped, altered or monoclonal antibody of this
invention in combination with at least one additional
20   monoclonal, altered or reshaped antibody. A particular
embodiment is provided in which the additional antibody
is an anti-RSV antibody distinguished from the subject
antibody of the invention by virtue of being reactive
with a different epitope of the RSV F protein antigen
25   than the subject antibody of the invention.

In a further aspect, the present invention provides
a method for passive immunotherapy of RSV disease in a
human by administering to said human an effective amount
of the pharmaceutical composition of the invention for
30   the prophylactic or therapeutic treatment of RSV
infection.

In yet another aspect, the present invention
provides methods for, and components useful in, the
recombinant production of human and altered antibodies

(e.g., engineered antibodies, CDRs, Fab or F(ab)$_2$ fragments, or analogs thereof) which are derived from human neutralizing monoclonal antibodies (mAbs) for the F protein of RSV. These components include isolated

5   nucleic acid sequences encoding same, recombinant plasmids containing the nucleic acid sequences under the control of selected regulatory sequences which are capable of directing the expression thereof in host cells (preferably mammalian) transfected with the

10   recombinant plasmids. The production method involves culturing a transfected host cell line of the present invention under conditions such that the human or altered antibody is expressed in said cells and isolating the expressed product therefrom.

15   In still another aspect of the invention is a method to diagnose the presence of RSV in a human which comprises contacting a sample of biological fluid with the human antibodies and altered antibodies and fragments thereof of the instant invention and assaying

20   for the occurrence of binding between said human antibody (or altered antibody, or fragment) and RSV.

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

25

Brief Description of the Drawings

Fig. 1A is a graph illustrating the competition of Gλ-1 scFV phage binding with RSV19 mAb [International patent publication No. WO92/04381, published March 19,

30   1992].

Fig. 1B is a graph illustrating the competition of Gλ-1 scFV phage binding with RSV B4 mAb [International patent publication No. WO93/20210, published October 14, 1993].

Fig. 2 is a graph illustrating virus neutralization by scFV phages, Gλ-1, Gλ-3, and G$_K$-1 with RSV strain 273.

Fig. 3 illustrates the DNA sequence [SEQ ID NO: 1] and protein sequence (amino acids reported in single

5   letter code) [SEQ ID NO: 2] for the Gλ-1 light chain variable region, processed N-terminus through framework IV.

Fig. 4 illustrates the DNA sequence [SEQ ID NO: 3] and protein sequence (amino acids reported in single

10   letter code) [SEQ ID NO: 4] for the Gλ-1 heavy chain variable region, processed N-terminus through framework IV.

Fig. 5 illustrates the cloning strategy used for the construction of the Gλ-1 monoclonal antibody. The

15   heavy chain V region was cloned into the pCD derivative vector as a XhoI - ApaI fragment. The entire light chain V region was cloned into the pCN derivative vector, 43-1pcn, as a SacI - AvrII fragment. Details are described below.

20   Fig. 6 provides a comparison of the heavy chain amino acid sequences of the Gλ-1 single chain F$_v$ [SEQ ID NO: 5] and various monoclonal antibodies of this invention. The amino acid sequences of the heavy chains for the A [SEQ ID NO: 7] and B [SEQ ID NO: 8] constructs

25   are shown. Numbering of the residues is based on the germline (GL) gene Dp58 [SEQ ID NO: 6], beginning at the mature processed amino terminus and ending at CDR3. The "-" indicates identity to the preceding sequence (eg., A compared to B). Bold residues correspond to the leader

30   region, and to CDRs 1-3.

Fig. 7 provides a comparison of the light chain amino acid sequences of the Gλ-1A single chain F$_v$ [SEQ ID NO: 9] and various monoclonal antibodies of this

invention. The amino acid sequences of the light chains
for the A [SEQ ID NO: 11] and B [SEQ ID NO: 12]
constructs are shown. Numbering of the residues in the
Vκ region is based on the germline (GL) gene DpL8 [SEQ
5   ID NO: 10], beginning at the mature processed amino
terminus and ending at CDR3. For reference to framework
4, the actual numbering is also shown for Gλ-1A. As in
Fig. 6, the "-" indicates identity to the preceding
sequence.

10   Figs. 8A to 8F illustrate the continuous DNA
sequence [SEQ ID NO: 13] of the expression plasmid Gλ-
1Apcd containing the RSV neutralizing human Gλ-1 mAb for
the heavy chain. The start of translation, leader
peptide, amino-terminal processing site, carboxy
15   terminus of the Gλ-1 heavy chain, and Eco RI restriction
endonuclease cleavage site are shown.

          Figs. 9A to 9E illustrate the continuous DNA
sequence [SEQ ID NO: 14] of the expression plasmid Gλ-
1Apcn containing the RSV neutralizing human Gλ-1 mAb for
20   the light chain. The corresponding features for the
light chain as for Figs. 8A-8F are shown.

          Figs. 10A and 10B illustrate the continuous DNA
sequence [SEQ ID NO: 15] of the coding region of the
heavy chain of plasmid Gλ-1Bpcd. Bolded residues
25   indicate differences from the full vector sequence for
Gλ-1Apcd in Figs. 8A-8F [SEQ ID NO: 13].

          Fig. 11 is the DNA sequence [SEQ ID NO: 16] of the
coding region for the light chain of plasmid Gλ-1Bpcn.
Bolded residues indicate differences from the full
30   vector sequence for Gλ-1Apcn in Figs. 9A-9E [SEQ ID NO:
14].

Detailed Description of the Invention

This invention provides useful human monoclonal
antibodies (and fragments thereof) reactive with the F
protein of RSV, isolated nucleic acids encoding same and
5   various means for their recombinant production as well
as therapeutic, prophylactic and diagnostic uses of such
antibodies and fragments thereof.

I.   Definitions.

As used in this specification and the claims, the
10   following terms are defined as follows:

"Altered antibody" refers to a protein encoded by
an altered immunoglobulin coding region, which may be
obtained by expression in a selected host cell. Such
altered antibodies are engineered antibodies (e.g.,
15   chimeric, humanized, or reshaped or immunologically
edited human antibodies) or fragments thereof lacking
all or part of an immunoglobulin constant region, e.g.,
Fv, Fab, or F(ab')$_2$ and the like.

"Altered immunoglobulin coding region" refers to a
20   nucleic acid sequence encoding an altered antibody of
the invention or a fragment thereof.

"Reshaped human antibody" refers to an altered
antibody in which minimally at least one CDR from a
first human monoclonal donor antibody is substituted for
25   a CDR in a second human acceptor antibody. Preferrably
all six CDRs are replaced. More preferrably an entire
antigen combining region (e.g., Fv, Fab or F(ab')$_2$) from
a first human donor monoclonal antibody is substituted
for the corresponding region in a second human acceptor
30   monoclonal antibody. Most preferrably the Fab region
from a first human donor is operatively linked to the
appropriate constant regions of a second human acceptor
antibody to form a full length monoclonal antibody.

"First immunoglobulin partner" refers to a nucleic
acid sequence encoding a human framework or human
immunoglobulin variable region in which the native (or
naturally-occurring) CDR-encoding regions are replaced
5   by the CDR-encoding regions of a donor human antibody.
The human variable region can be an immunoglobulin heavy
chain, a light chain (or both chains), an analog or
functional fragments thereof. Such CDR regions, located
within the variable region of antibodies
10   (immunoglobulins) can be determined by known methods in
the art. For example, Kabat *et al.* (Sequences of
Proteins of Immunological Interest, 4th Ed., U.S.
Department of Health and Human Services, National
Institutes of Health (1987)) disclose rules for locating
15   CDRs. In addition, computer programs are known which
are useful for identifying CDR regions/structures.

     "Second fusion partner" refers to another
nucleotide sequence encoding a protein or peptide to
which the first immunoglobulin partner is fused in frame
20   or by means of an optional conventional linker sequence
(i.e., operatively linked). Preferably the fusion
partner is an immunoglobulin gene and when so, it is
referred to as a "second immunoglobulin partner". The
second immunoglobulin partner may include a nucleic acid
25   sequence encoding the entire constant region for the
same (i.e., homologous - the first and second altered
antibodies are derived from the same source) or an
additional (i.e., heterologous) antibody of interest.
It may be an immunoglobulin heavy chain or light chain
30   (or both chains as part of a single polypeptide). The
second immunoglobulin partner is not limited to a
particular immunoglobulin class or isotype. In
addition, the second immunoglobulin partner may comprise
part of an immunoglobulin constant region, such as found

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in a Fab, or F(ab)$_2$ (i.e., a discrete part of an
appropriate human constant region or framework region).

A second fusion partner may also comprise a sequence
encoding an integral membrane protein exposed on the
5   outer surface of a host cell, e.g., as part of a phage
display library, or a sequence encoding a protein for
analytical or diagnostic detection, e.g., horseradish
peroxidase (HRP), β-galactosidase, etc.

The terms Fv, Fc, Fd, Fab, or F(ab')$_2$ are used with
10   their standard meanings [see, e.g., Harlow *et al.*,
Antibodies A Laboratory Manual, Cold Spring Harbor
Laboratory, (1988)].

As used herein, an "engineered antibody" describes
a type of altered antibody, i.e., a full-length
15   synthetic antibody (e.g., a chimeric, humanized,
reshaped or immunologically edited human antibody as
opposed to an antibody fragment) in which a portion of
the light and/or heavy chain variable domains of a
selected acceptor antibody are replaced by analogous
20   parts from one or more donor antibodies which have
specificity for the selected epitope. For example, such
molecules may include antibodies characterized by a
humanized heavy chain associated with an unmodified
light chain (or chimeric light chain), or vice versa.
25   Engineered antibodies may also be characterized by
alteration of the nucleic acid sequences encoding the
acceptor antibody light and/or heavy variable domain
framework regions in order to retain donor antibody
binding specificity. These antibodies can comprise
30   replacement of one or more CDRs (preferably all) from
the acceptor antibody with CDRs from a donor antibody
described herein.

A "chimeric antibody" refers to a type of
engineered antibody which contains naturally-occurring

variable region (light chain and heavy chains) derived
from a donor antibody in association with light and
heavy chain constant regions derived from an acceptor
antibody from a heterologous species.

5   A "humanized antibody" refers to a type of
engineered antibody having its CDRs derived from a non-
human donor immunoglobulin, the remaining
immunoglobulin-derived parts of the molecule being
derived from one (or more) human immunoglobulin(s). In
10   addition, framework support residues may be altered to
preserve binding affinity [see, e.g., Queen *et al.*,
Proc. Nat'l. Acad. Sci. USA, 86:10029-10032 (1989),
Hodgson *et al.*, Bio/Technology, 9:421 (1991)].

       An "immunologically edited antibody" refers to a
15   type of engineered antibody in which changes are made in
donor and/or acceptor sequences to edit regions in
respect of cloning artifacts, germ line enhancements,
etc. aimed at reducing the likelihood of an
immunological response to the antibody on the part of a
20   patient being treated with the edited antibody.

       The term "donor antibody" refers to an antibody
(monoclonal, or recombinant) which contributes the
nucleic acid sequences of its variable regions, CDRs, or
other functional fragments or analogs thereof to a first
25   immunoglobulin partner, so as to provide the altered
immunoglobulin coding region and resulting expressed
altered antibody with the antigenic specificity and
neutralizing activity characteristic of the donor
antibody. One donor antibody suitable for use in this
30   invention is a Fab fragment of a human neutralizing
monoclonal antibody designated as Fab Gλ-1. Fab Gλ-1 is
defined as a having the variable light and heavy chain
DNA and amino acid sequences Gλ-1 as shown in Figs. 3,
4, 8A-8F and 9A-9E [SEQ ID NOS: 1-4, 13 and 14].

The term "acceptor antibody" refers to an antibody
(monoclonal or recombinant) from a source genetically
unrelated to the donor antibody, which contributes all
(or any portion, but preferably all) of the nucleic acid
5   sequences encoding its heavy and/or light chain
framework regions and/or its heavy and/or light chain
constant regions to the first immunoglobulin partner.
Preferably a human antibody is the acceptor antibody.
     "CDRs" are defined as the complementarity
10   determining region amino acid sequences of an antibody
which are the hypervariable regions of immunoglobulin
heavy and light chains [see, e.g., Kabat *et al.*,
Sequences of Proteins of Immunological Interest, 4th
Ed., U.S. Department of Health and Human Services,
15   National Institutes of Health (1987)]. There are three
heavy chain and three light chain CDRs (or CDR regions)
in the variable portion of an immunoglobulin. Thus,
"CDRs" as used herein refers to all three heavy chain
CDRs, or all three light chain CDRs (or both all heavy
20   and all light chain CDRs, if appropriate). CDRs provide
the majority of contact residues for the binding of the
antibody to the antigen or epitope. CDRs of interest in
this invention are derived from donor antibody variable
heavy and light chain sequences, and include analogs of
25   the naturally occurring CDRs, which analogs also share
or retain the same antigen binding specificity and/or
neutralizing ability as the donor antibody from which
they were derived.
     By "sharing the antigen binding specificity or
30   neutralizing ability" is meant, for example, that
although Fab Gλ-1 may be characterized by a certain
level of antigen affinity, a CDR encoded by a nucleic
acid sequence of Fab Gλ-1 in an appropriate structural
environment may have a lower, or higher affinity. It is

expected that CDRs of Fab Gλ-1 in such environments will nevertheless recognize the same epitope(s) as does the intact Fab Gλ-1. A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor

5   deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or neutralizing ability as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence modified by

10   at least one amino acid, wherein said modification can be a chemical modification, or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen

15   specificity and high affinity, of the unmodified sequence. For example, (silent) mutations can be constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions.

20   Analogs may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid or peptide sequences of the invention. Such variations or modifications may be due to degeneracy in the genetic

25   code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein

30   carrier molecules to which the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used

in the diagnostic field, e.g., polystyrene or other
plastic beads, polysaccharides, e.g., as used in the
BIAcore (Pharmacia) system, or other non-protein
substances useful in the medical field and safe for
administration to humans and animals. Other effector
agents may include a macrocycle, for chelating a heavy
metal atom, or radioisotopes. Such effector agents may
also be useful to increase the half-life of the altered
antibodies, e.g., polyethylene glycol.

        II. *Combinatorial Cloning.*

        As mentioned above, a number of problems have
hampered the direct application of the hybridoma
technology [G. Kohler and C. Milstein, Nature, 256: 495-
497 (1975)] to the generation and isolation of human
monoclonal antibodies. Among these are a lack of
suitable fusion partner myeloma cell lines used to form
hybridoma cell lines as well as the poor stability of
such hybridomas even when formed. These shortcomings
are further exacerbated in the case of RSV because of
the paucity of viral specific B cells in the peripheral
circulation. Therefore, the molecular biological
approach of combinatorial cloning is preferred.

        Combinatorial cloning is disclosed generally in PCT
Publication No. WO90/14430. Simply stated, the goal of
combinatorial cloning is to transfer to a population of
bacterial cells the immunological genetic capacity of a
human cell, tissue or organ. It is preferred to employ
cells, tissues or organs which are immunocompetent.
Particularly useful sources include, without limitation,
spleen, thymus, lymph nodes, bone marrow, tonsil and
peripheral blood lymphocytes. The cells may be
optionally RSV stimulated *in vitro,* or selected from
donors which are known to have produced an immune
response or donors who are HIV⁺ but asymptomatic.

The genetic information isolated from the donor
cells can be in the form of DNA or RNA and is
conveniently amplified by Polymerase Chain Reaction
(PCR) or similar techniques. When isolated as RNA the
5   genetic information is preferably converted into cDNA by
reverse transcription prior to amplification. The
amplification can be generalized or more specifically
tailored. For example, by a careful selection of PCR
primer sequences, selective amplification of
10   immunoglobulin genes or subsets within that class of
genes can be achieved.

Once the component gene sequences are obtained, in
this case the genes encoding the variable regions of the
various heavy and light antibody chains, the light and
15   heavy chain genes are associated in random combinations
to form a random combinatorial library. Various
recombinant DNA vector systems have been described to
facilitate combinatorial cloning [see: PCT Publication
No. WO90/14430 supra; Scott and Smith, Science 249:386-
20   406 (1990); or U. S. Patent 5,223,409]. Having
generated the combinatorial library, the products can,
after expression, be conveniently screened by biopanning
with RSV F protein or, if necessary, by epitope blocked
biopanning as described in more detail below.

25   As described herein, it is preferred to use single
chain antibodies for combinatorial cloning and screening
and then to convert them to full length mAbs after
selection of the desired candidate molecules. However,
Fab fragments of mAbs can also be used for cloning and
30   screening.

III. Antibody Fragments.

The present invention contemplates the use of scFv,
Fab, or F(ab')$_2$ fragments to derived full-length mAbs
directed against the F protein of RSV. Although these

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fragments may be independently useful as protective and
therapeutic agents *in vivo* against RSV-mediated
conditions or *in vitro* as part of an RSV diagnostic,
they are employed herein as a component of a reshaped

5   human antibody. A scFv fragment contains the light and
heavy chain variable regions joined by a linker of about
12 amino acids in either a light-linker-heavy or a
heavy-linker-light orientation. A Fab fragment contains
the entire light chain and amino terminal portion of the

10   heavy chain; and a F(ab')$_2$ fragment is the fragment
formed by two Fab fragments bound by additional
disulfide bonds. RSV binding monoclonal antibodies
provide sources of scFv or Fab fragments which can be
obtained from a combinatorial phage library [see, e.g.,

15   Winter *et al.*, Ann. Rev. Immunol., 12:433-455 (1994) or
Barbas *et al.*, Proc. Nat'l. Acad. Sci. (USA) 89, 10164-
10168 (1992), which are both hereby incorporated by
reference in their entireties].

            IV.   Anti-RSV Antibody Amino Acid and Nucleotide
20   *Sequences of Interest.*

            The Fab Gλ-1 or other antibodies described herein
may contribute sequences, such as variable heavy and/or
light chain peptide sequences, framework sequences, CDR
sequences, functional fragments, and analogs thereof,

25   and the nucleic acid sequences encoding them, useful in
designing and obtaining various altered antibodies which
are characterized by the antigen binding specificity of
the donor antibody.

            As one example, the present invention thus provides

30   variable light chain and variable heavy chain sequences
from the RSV human Fab Gλ-1A and sequences derived
therefrom. The heavy chain variable region of Fab Gλ-1A
is illustrated by Figs. 4, 8A-8F and 10A-10B [SEQ ID
NOS: 3-4, 13 and 15].

The nucleic acid sequences of this invention, or
fragments thereof, encoding the variable light chain and
heavy chain peptide sequences are also useful for
mutagenic introduction of specific changes within the

5   nucleic acid sequences encoding the CDRs or framework
regions, and for incorporation of the resulting modified
or fusion nucleic acid sequence into a plasmid for
expression. For example, silent substitutions in the
nucleotide sequence of the framework and CDR-encoding

10   regions can be used to create restriction enzyme sites
which would facilitate insertion of mutagenized CDR
(and/or framework) regions. These CDR-encoding regions
may be used in the construction of reshaped human
antibodies of this invention.

15   Taking into account the degeneracy of the genetic
code, various coding sequences may be constructed which
encode the variable heavy and light chain amino acid
sequences, and CDR sequences of the invention as well as
functional fragments and analogs thereof which share the

20   antigen specificity of the donor antibody. The isolated
nucleic acid sequences of this invention, or fragments
thereof, encoding the variable chain peptide sequences
or CDRs can be used to produce altered antibodies, e.g.,
chimeric or humanized antibodies, or other engineered

25   antibodies of this invention when operatively combined
with a second immunoglobulin partner.
         It should be noted that in addition to isolated
nucleic acid sequences encoding portions of the altered
antibody and antibodies described herein, other such

30   nucleic acid sequences are encompassed by the present
invention, such as those complementary to the native
CDR-encoding sequences or complementary to the human
framework regions surrounding the CDR-encoding regions.
Such sequences include all nucleic acid sequences which

by virtue of the redundancy of the genetic code are capable of encoding the same amino acid sequence as given in Figs. 3 and 4 [SEQ ID NOS: 2 and 4]. Figs. 6 and 7 [SEQ ID NOS: 5-12] provide representations of such

5 sequences. Other useful DNA sequences encompassed by this invention include those sequences which hybridize under stringent hybridization conditions [See: T. Maniatis *et al.*, <u>Molecular Cloning (A Laboratory Manual)</u>, Cold Spring Harbor Laboratory (1982), pages 387

10 to 389] to the DNA sequences encoding the Gλ-1 antibodies (e.g., sequences of Figs. 3, 4, 8A-8F through 11 [SEQ ID NOS: 1-4, 13-16]) and which retain the antigen binding properties of those antibodies. An example of one such stringent hybridization condition is

15 hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for an hour. Alternatively an exemplary stringent hybridization condition is in 50% formamide, 4XSSC at 42°C. Preferably, these hybridizing DNA sequences are at least about 18 nucleotides in length,

20 i.e., about the size of a CDR.

   V.   Altered Immunoglobulin Coding Regions and Altered Antibodies.

   Altered immunoglobulin coding regions encode altered antibodies which include engineered antibodies

25 such as chimeric antibodies, humanized, reshaped, and immunologically edited human antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions in the form of scFv regions that encode peptides having the antigen specificity of an RSV

30 antibody, preferably a high affinity antibody such as provided by the present invention, inserted into an acceptor immunoglobulin partner.

   When the acceptor is an immunoglobulin partner, as defined above, it includes a sequence encoding a second

antibody region of interest, for example, an Fc region. Immunoglobulin partners may also include sequences encoding another immunoglobulin to which the light or heavy chain constant region is fused in frame or by

5 means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of RSV may be designed to elicit enhanced binding with the same antibody.

The immunoglobulin partner may also be associated

10 with effector agents as defined above, including non-protein carrier molecules, to which the immunoglobulin partner may be operatively linked by conventional means.

Fusion or linkage between the immunoglobulin partners, e.g., antibody sequences, and the effector

15 agent may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques are known in the art and readily described in

20 conventional chemistry and biochemistry texts.

Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin

25 coding region. The design of such linkers is well known to those of skill in the art.

In addition, signal sequences for the molecules of the invention may be modified to enhance expression. For example the reshaped human antibody having the

30 signal sequence and CDRs derived from the Fab Gλ-1 heavy chain sequence, may have the original signal peptide replaced with another signal sequence such as the Campath leader sequence [Page, M. J. *et al.*, BioTechnology 9:64-68(1991)].

An exemplary altered antibody, a reshaped human antibody, contains a variable heavy and the entire light chain peptide or protein sequence having the antigen specificity of Fab Gλ-1, fused to the constant heavy

5   regions C_{H-1}-C_{H-3} derived from a second human antibody.

In still a further embodiment, the engineered antibody of the invention may have attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an

10   engineered antibody of the invention in which the Fc fragment or $C_{H-2}C_{H-3}$ domain of a complete antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

15   Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any discrete fragment thereof, such as the Fab or F(ab')$_2$ fragments, a heavy chain dimer, or any minimal recombinant fragments

20   thereof such as an F_v or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor Fab Gλ-1. Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

25   Whenever the immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise immunoglobulin (Ig) constant

30   regions and variable framework regions from one source, e.g., the acceptor antibody, and one or more (preferably all) CDRs from the donor antibody, e.g., the anti-RSV antibody described herein. In addition, alterations, e.g., deletions, substitutions, or additions, of the

acceptor mAb light and/or heavy variable domain
framework region at the nucleic acid or amino acid
levels, or the donor CDR regions may be made in order to
retain donor antibody antigen binding specificity or to
5   reduce potential immunogenicity.

Such engineered antibodies are designed to employ
one (or both) of the variable heavy and/or light chains
of the RSV mAb (optionally modified as described) or one
or more of the below-identified heavy or light chain
10   CDRs. The engineered antibodies of the invention are
neutralizing, i.e., they desirably inhibit virus growth
in vitro and *in vivo* in animal models of RSV infection.

Such engineered antibodies may include a reshaped
human antibody containing the human heavy and light
15   chain constant regions fused to the RSV antibody
functional fragments. A suitable human (or other
animal) acceptor antibody may be one selected from a
conventional database, e.g., the KABAT® database, Los
Alamos database, and Swiss Protein database, by homology
20   to the nucleotide and amino acid sequences of the donor
antibody. A human antibody characterized by a homology
to the framework regions of the donor antibody (on an
amino acid basis) may be suitable to provide a heavy
chain constant region and/or a heavy chain variable
25   framework region for insertion of the donor CDRs. A
suitable acceptor antibody capable of donating light
chain constant or variable framework regions may be
selected in a similar manner. It should be noted that
the acceptor antibody heavy and light chains are not
30   required to originate from the same acceptor antibody.

Desirably the heterologous framework and constant
regions are selected from human immunoglobulin classes
and isotypes, such as IgG (subtypes 1 through 4), IgM,
IgA and IgE. The Fc domains are not limited to native

sequences, but include mutant variants known in the art
that alter function. For example, mutations have been
described in the Fc domains of certain IgG antibodies
that reduce Fc-mediated complement and Fc receptor

5   binding [see, e.g., A. R. Duncan et al., Nature,
332:563-564 (1988); A. R. Duncan and G. Winter, Nature,
332:738-740 (1988); M.-L. Alegre et al., J. Immunol.,
148:3461-3468 (1992); M.-H. Tao et al., J. Exp. Med.,
178:661-667 (1993); and V. Xu et al. J. Biol. Chem.,

10 269:3469-2374 (1994)]; alter clearance rate [J.-K. Kim
et al., Eur. J. Immunol., 24:542-548 (1994)]; and reduce
structural heterogeneity [S. Angal et al., Mol. Immunol.
30:105-108 (1993)]. Also, other modifications are
possible such as oligomerization of the antibody by

15 addition of the tailpiece segment of IgM and other
mutations [R. I. F. Smith and S. L. Morrison,
Biotechnology 12:683-688 (1994); R. I. F. Smith et al.,
J. Immunol., 154: 2226-2236 (1995)] or addition of the
tailpiece segment of IgA [I. Kariv et al., J. Immunol.,

20 157: 29-38 (1996)]. However, the acceptor antibody need
not comprise only human immunoglobulin protein
sequences. For instance a gene may be constructed in
which a DNA sequence encoding part of a human
immunoglobulin chain is fused to a DNA sequence encoding

25 a non-immunoglobulin amino acid sequence such as a
polypeptide effector or reporter molecule.

The altered antibody thus preferably has the
structure of a natural human antibody or a fragment
thereof, and possesses the combination of properties

30 required for effective therapeutic use, e.g., treatment
of RSV mediated diseases in man, or for diagnostic uses.

It will be understood by those skilled in the art
that an altered antibody may be further modified by
changes in variable domain amino acids without

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necessarily affecting the specificity and high affinity
of the donor antibody (i.e., an analog). It is
anticipated that heavy and light chain amino acids may
be substituted by other amino acids either in the

5   variable domain frameworks or CDRs or both.
Particularly preferred is the immunological editing of
such reconstructed sequences as illustrated in the
examples herein.

In addition, the variable or constant region may be

10   altered to enhance or decrease selective properties of
the molecules of the instant invention, as described
above. For example, dimerization, binding to Fc
receptors, or the ability to bind and activate
complement [see, e.g., Angal *et al.*, Mol. Immunol,

15   30:105-108 (1993); Xu *et al.*, J. Biol. Chem, 269:3469-
3474 (1994); and Winter *et al.*, EP 307,434-B].

Such antibodies are useful in the prevention and
treatment of RSV mediated disorders, as discussed below.

VI. Production of Altered antibodies and

20   *Engineered Antibodies.*

The resulting reshaped human antibodies of this
invention can be expressed in recombinant host cells,
e.g., COS, CHO or myeloma cells. A conventional
expression vector or recombinant plasmid is produced by

25   placing these coding sequences for the altered antibody
in operative association with conventional regulatory
control sequences capable of controlling the replication
and expression in, and/or secretion from, a host cell.
Regulatory sequences include promoter sequences, e.g.,

30   CMV promoter, and signal sequences, which can be derived
from other known antibodies. Similarly, a second
expression vector can be produced having a DNA sequence
which encodes a complementary antibody light or heavy
chain. Preferably this second expression vector is

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identical to the first except insofar as the coding
sequences and selectable markers are concerned. This
ensures as far as possible that each polypeptide chain
is functionally expressed. Alternatively, the heavy and
5   light chain coding sequences for the altered antibody
may reside on a single vector.

A selected host cell is co-transfected by
conventional techniques with both the first and second
vectors (or simply transfected by a single vector) to
10   create the transfected host cell of the invention
comprising both the recombinant or synthetic light and
heavy chains. The transfected cell is then cultured by
conventional techniques to produce the engineered
antibody of the invention. The production of the
15   antibody which includes the association of both the
recombinant heavy chain and light chain is measured in
the culture by an appropriate assay, such as an enzyme-
linked immunosorbent assay (ELISA) or radioimmunoassay
(RIA). Similar conventional techniques may be employed
20   to construct other altered antibodies and molecules of
this invention.

Suitable vectors for the cloning and subcloning
steps employed in the methods and construction of the
compositions of this invention may be selected by one of
25   skill in the art. For example, the conventional pUC
series of cloning vectors, may be used. One vector used
is pUC19, which is commercially available from supply
houses, such as Amersham (Buckinghamshire, United
Kingdom) or Pharmacia (Uppsala, Sweden). Any vector,
30   which is capable of replicating readily, has an
abundance of cloning sites and selectable genes (e.g.,
antibiotic resistance), and is easily manipulated, may
be used for cloning. Thus, the selection of the cloning
vector is not a limiting factor in this invention.

Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vectors. Preferred vectors include for

5   example plasmids pCD or pCN. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above described DNA sequences

10   which code for the engineered antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

15   The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a polyadenylation (polyA)

20   signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art. The components of such vectors, e.g. replicons,

25   selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other

30   appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

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The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or altered immunoglobulin molecules thereof. Host cells useful for
5   the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this
10   invention.

Suitable host cells or cell lines for the expression of the engineered antibody or altered antibody of the invention are preferably mammalian cells such as CHO, COS, a fibroblast cell (e.g., 3T3), and
15   myeloid cells, and more preferably a CHO or a myeloid cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian
20   host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook *et al.*, Molecular Cloning (A Laboratory Manual), 2nd edit., Cold Spring Harbor Laboratory (1989).

25   Bacterial cells may prove useful as host cells suitable for the expression of the recombinant scFvs, Fabs and MAbs of the present invention [see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)]. The tendency of proteins expressed in bacterial cells to be
30   in an unfolded or improperly folded form or in a non-glycosylated form does not pose as great a concern because Fabs are not normally glycosylated and can be engineered for exported expression, thereby reducing the high concentration that facilitates misfolding.

Nevertheless, any recombinant Fab produced in a bacterial cell would be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced and exported in a

5   properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be

10   employed in this method.

      Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems [see, e.g.

15   Miller *et al.*, Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein].

      The general methods by which the vectors of the invention may be constructed, the transfection methods required to produce the host cells of the invention, and

20   culture methods necessary to produce the altered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the altered antibodies of the invention may be purified from the cell culture contents according to standard

25   procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention.

30   Yet another method of expression of reshaped antibodies may utilize expression in a transgenic animal. An exemplary systems is described in U. S. Patent No. 4,873,316. The expression system described in that reference uses the animal's casein promoter and,

when transgenically incorporated into a mammal, permits
the female to produce the desired recombinant protein in
its milk.

5   Once expressed by the desired method, the
engineered antibody is then examined for *in vitro*
activity by use of an appropriate assay. At present,
conventional ELISA assay formats are employed to assess
qualitative and quantitative binding of the altered
antibody to RSV. Additionally, other *in vitro* assays
10   and *in vivo* animal models may also be used to verify
neutralizing efficacy prior to subsequent human clinical
studies performed to evaluate the persistence of the
altered antibody in the body despite the usual clearance
mechanisms.

15   *VII. Therapeutic/Prophylactic Uses.*

This invention also relates to a method of treating
humans experiencing RSV-related symptoms which comprises
administering an effective dose of antibodies including
one or more of the antibodies (altered, reshaped,
20   monoclonal, etc.) described herein or fragments thereof.

The therapeutic response induced by the use of the
molecules of this invention is produced by binding to
RSV and thus subsequently blocking RSV propagation.
Thus, the molecules of the present invention, when in
25   preparations and formulations appropriate for
therapeutic use, are highly desirable for those persons
experiencing RSV infection. For example, longer
treatments may be desirable when treating seasonal
episodes or the like. The dose and duration of
30   treatment relates to the relative duration of the
molecules of the present invention in the human
circulation, and can be adjusted by one of skill in the
art depending upon the condition being treated and the
general health of the patient.

The altered antibodies, antibodies and fragments
thereof of this invention may also be used alone or in
conjunction with other antibodies, particularly human or
humanized mAbs reactive with other epitopes on the F
5   protein or other RSV target antigens as prophylactic
agents.

The mode of administration of the therapeutic and
prophylactic agents of the invention may be any suitable
route which delivers the agent to the host. The altered
10   antibodies, antibodies, engineered antibodies, and
fragments thereof, and pharmaceutical compositions of
the invention are particularly useful for parenteral
administration, i.e., subcutaneously, intramuscularly,
intravenously, or intranasally.

15   Therapeutic and prophylactic agents of the
invention may be prepared as pharmaceutical compositions
containing an effective amount of the altered antibody
of the invention as an active ingredient in a
pharmaceutically acceptable carrier. An aqueous
20   suspension or solution containing the antibody,
preferably buffered at physiological pH, in a form ready
for injection is preferred. The compositions for
parenteral administration will commonly comprise a
solution of the engineered antibody of the invention or
25   a cocktail thereof dissolved in an pharmaceutically
acceptable carrier, preferably an aqueous carrier. A
variety of aqueous carriers may be employed, e.g., 0.4%
saline, 0.3% glycine, and the like. These solutions are
sterile and generally free of particulate matter. These
30   solutions may be sterilized by conventional, well known
sterilization techniques (e.g., filtration). The
compositions may contain pharmaceutically acceptable
auxiliary substances as required to approximate
physiological conditions such as pH adjusting and

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buffering agents, etc. The concentration of the
antibody of the invention in such pharmaceutical
formulation can vary widely, i.e., from less than about
0.5%, usually at or at least about 1% to as much as 15
5   or 20% by weight and will be selected primarily based on
fluid volumes, viscosities, etc., according to the
particular mode of administration selected.

Thus, a pharmaceutical composition of the invention
for intramuscular injection could be prepared to contain
10   1 mL sterile buffered water, and between about 1 ng to
about 100 mg, e.g. about 50 ng to about 80 mg, or more
preferably, about 5 mg to about 75 mg, of an engineered
antibody of the invention. Similarly, a pharmaceutical
composition of the invention for intravenous infusion
15   could be made up to contain about 250 ml of sterile
Ringer's solution, and about 1 to about 75 and
preferably 5 to about 50 mg/ml of an engineered antibody
of the invention. Actual methods for preparing
parenterally administrable compositions are well known
20   or will be apparent to those skilled in the art and are
described in more detail in, for example, Remington's
Pharmaceutical Science, 15th ed., Mack Publishing
Company, Easton, Pennsylvania.

It is preferred that the therapeutic and
25   prophylactic agents of the invention, when in a
pharmaceutical preparation, be present in unit dose
forms. The appropriate therapeutically effective dose
can be determined readily by those of skill in the art.
To effectively treat an inflammatory disorder in a human
30   or other animal, one dose of approximately 0.1 mg to
approximately 20 mg per 70 kg body weight of a protein
or an antibody of this invention should be administered
parenterally, preferably i.v. or i.m. (intramuscularly).

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Such dose may, if necessary, be repeated at appropriate
time intervals selected as appropriate by a physician.

The altered antibodies and engineered antibodies of
this invention may also be used in diagnostic regimens,
5   such as for the determination of RSV mediated disorders
or tracking progress of treatment of such disorders. As
diagnostic reagents, these altered antibodies may be
conventionally labeled for use in ELISAs and other
conventional assay formats for the measurement of RSV
10   levels in serum, plasma or other appropriate tissue, or
the release by human cells in culture. The nature of
the assay in which the altered antibodies are used are
conventional and do not limit this disclosure.

The antibodies, altered antibodies or fragments
15   thereof described herein can be lyophilized for storage
and reconstituted in a suitable carrier prior to use.
This technique has been shown to be effective with
conventional immunoglobulins and art-known
lyophilization and reconstitution techniques can be
20   employed.

The following examples illustrate various aspects
of this invention including the construction of
exemplary engineered antibodies and expression thereof
in suitable vectors and host cells, and are not to be
25   construed as limiting the scope of this invention. All
amino acids are identified by conventional three letter
or single letter codes. All necessary restriction
enzymes, plasmids, and other reagents and materials were
obtained from commercial sources unless otherwise
30   indicated. All general cloning ligation and other
recombinant DNA methodology were as performed in T.
Maniatis *et al.*, cited above, or Sambrook *et al.*, cited
above.

Example 1:   Isolation of Gλ-1 scFv-1

Single chain (sc) Fv libraries were prepared from
an individual purposely exposed to RSV and selected
against recombinant RSV F-protein following described
5   procedures [R. H. Jackson *et al*, in *Protein Engineering,
A Practical Approach*, A. R. Rees *et al* eds, Oxford
University Press, chapter 12, pp. 277-301, 1992; H. R.
Hoogenboom *et al.*, <u>Nucl. Acid Res.</u>, 19: 4133-4137
(1991); J. D. Marks *et al.*, <u>J. Mol. Biol.</u>, 222: 581-597
10   (1991)].   Briefly, lymphocytes were isolated from a
blood sample taken 15 days post exposure.   RNA isolated
from the lymphocytes was used for preparation of scFv
encoding repertoires for phage display.   Sets of V-
region primers were paired with constant region primers
15   for heavy chain domain 1 IgG and IgM and light chain C-κ
and C-λ and then linked in a scFv VH-VL orientation with
a 15 amino acid spacer (glycine$_4$-serine)$_3$ [SEQ ID NO: 21]
by overlap PCR [see J. D. Marks *et al.*, cited above, for
description of the primers].

The resulting four scFv repertoires (V-κ with IgG
20   and IgM, V-λ with IgG and IgM) were cloned into a
phagemid vector similar to pHEN1 [H. R. Hoogenboom *et
al.*, cited above] resulting in fusion of the scFvs to
gene III of phage fd.   The vector was then transformed
25   into *E. coli* (e.g., strain TG1) by electroporation to
yield the corresponding phagemid libraries.

Phage libraries displaying the scFv-gene 3 fusions
were prepared by infection of each of the plasmid
libraries with the M13K07 helper phage [R. H. Jackson,
30   cited above] and were individually subjected to 2 rounds
of panning against recombinant F-protein coated onto
plastic.   In the first round, 10^{11} phage in 2.5 ml
phosphate buffered saline (PBS)/2% Marval™ non-fat dry

milk were incubated for 90 minutes in a tube coated with

5 µg/ml of F-protein [described in P. Tsui *et al*, J.

Immunol., 157:772-780 (1996)] followed by 1 wash with

10x PBS/0.05% Tween 20 and a second wash with 10x PBS

5   alone. Bound phage were eluted with 10 mM triethylamine

and the eluate was neutralized with 1 M Tris-HCl, pH

7.4. The eluted phage were amplified and subjected to a

similar second round of panning, except that the

concentration of F-protein for coating was 2 µg/ml and

10   the wash buffer contained 20x PBS.

     E. coli were infected with the eluted phage and 96

colonies from each starting library were superinfected

with helper phage and screened for F-protein binding

activity. Only four positive clones were obtained from

15   the 2 IgM libraries, whereas 41 positives were observed

for the IgG libraries. By partial sequence analysis,

all of the clones carried one of three different heavy

chains. Complete sequences were obtained for the heavy

and light chain V-regions for six clones, all from the

20   IgG libraries.

     Serial dilutions of titered phage stocks of each of

these six clones were tested by ELISA for binding to

recombinant F-protein and to RSV infected cell lysate.

All showed binding to F-protein with the phage

25   designated Gλ-1 showing the best activity. However, Gλ-

1 and three other clones showed little binding to the

RSV lysate.

     Three clones: Gλ-1, Gλ-3 (lysate binding

positive), and Gκ-1 (lysate binding negative), where "κ"

30   and "λ" designate the class of the light chain, were

characterized further for competition of their binding

by F-protein specific neutralizing monoclonal

antibodies, and their ability to inhibit virus

infection.   The neutralizing mAbs RSV19 and B4 described
in International patent publication No. WO92/04381,
published March 19, 1992, and International patent
publication No. WO93/20210, published October 14, 1993,

5   recognize distinct epitopes on the F-protein.   Gκ-1 was
strongly inhibited by both antibodies.   Gλ-1 was
significantly inhibited by B4 only.   Gκ-3 was not
inhibited by either antibody (shown for Gλ-1 only; see
Figs. 1A and 1B).   In initial assays (Table I,

10   experiments 1-3), all three clones showed neutralizing
activity *in vitro*, with Gλ-1 being the most potent (Fig.
2, a graph of experiment 2), while control wild-type
phage (M13K07) not displaying scFv had no effect.

   To address the possibility that neutralization

15   might result just from phage coating of virus,
irrespective of epitope, a phage preparation of the non-
neutralizing Fab 5-16 was tested in the same assay.   In
three out of four assays, this preparation also showed
good neutralization activity, as did the control phage

20   in two of these assays (Table I, experiments 4-7).   This
confounding observation of variable neutralization by
both Fab 5-16 and control M13K07 phage rendered the
viral neutralization studies inconclusive.

Table I

Phage Sample	Virus Neutralization (IC_{50} x 10^{-7})[1] (aru or kru/ml)[2]						
	Experiment #						
	1	2	3	4	5	6	7
GK-1 a	1,600		<300				
b				<10	<7		
Gλ-1 a		80	<300				
b				8.1	11		
c							120
Gλ-3 a		900	<300	180			
b					<7	10	
c							730
M13K07a			>10^5	>10^5		>5,000	
b					+all dil.	+all dil.	>10^5
Fab 5-19a				>10^5	40	180	
b							3.5

Legend:

[1]   Assay according to M. J. Cannon, J. Virol. Meth.,
        16:293-301. Virus at 100 infectious centers/well
        was incubated with dilutions of the indicated phage
        for 1 hr and then added to susceptible cells for 3
        hr. The virus/phage solution was aspirated and
        replaced with fresh medium and the cells were
        incubated overnight before peroxidase staining for
        virus infected cells.

[2]   aru = ampicillin resistance units, a measure of
        phagmid containing particles.

        kru = kanamycin resistance units, a measure of
        particles containing the phage genome (for the
        M13K07 control only).

In the face of these results, made more ambiguous
by the dependence of all assays on phage stocks verses
antibody proteins of known concentration, Gλ-1 was
selected as the most likely candidate for a potent
5   neutralizing antibody based on (1) its apparent better
binding to F-protein, (2) its selective inhibition of
binding by the B4 antibody, and (3) its suggested
activity over background in the virus neutralization
assay.
10

Example 2:   Conversion of Gλ-1 scFV to mAb Version A
The DNA and encoded protein sequences of the VH and
VL regions of Gλ-1 are shown in Figs. 3 [SEQ ID NOS: 1
and 2] and 4 [SEQ ID NOS: 3 and 4], respectively. For
15   expression in mammalian cells, the heavy chain variable
region and the light chain variable region from the Gλ-1
plasmid were cloned into derivatives of plasmid pCDN
[Nambi, A. *et al.*, Mol. Cell. Biochem., 131:75-86
(1994)] in which the expression of the antibody chain is
20   driven by the cytomegalovirus promoter (CMV) promoter.
Plasmid pCD-HC68B is used for expressing full length
heavy chains and plasmid pCN-HuLC, for expressing full
length light chains.
In the initial constructs, changes in the sequence
25   at the amino terminus were introduced by the PCR primers
used for cloning the light chain and heavy chain
variable regions from plasmid Gλ-1. In these
constructs, the peptide signal sequence for both the
heavy and light chains is derived from the Campath light
30   chain [M. J. Page *et al.*, Biotechnology 9: 64-68
(1991)]. The heavy chain of Gλ-1 was PCR amplified from
Gλ-1 phagemid DNA, using primers for the amino terminus
and framework 4 of the variable region. The resulting

PCR fragment was cut with XhoI (site introduced by the amino terminus primer) and BstEII (naturally occurring site in framework 4), and cloned into an intermediate vector, F4HCV, at the XhoI/BstEII sites.

5   This cloning grafted the variable region of Gλ-1 onto the constant region of another anti-RSV heavy chain 194-F4 [cloned at SmithKline Beecham from a human hybridoma]. This intermediate clone was cut with XhoI and Bsp120I, and introduced into the same sites in pCD-

10   HC68B. The XhoI site is introduced at the amino terminus by the PCR primer and, when cloned into pCD-HC68B at the same site is preceded in frame by the Campath leader sequence. The Bsp120I site is a naturally occurring, highly conserved sequence at the

15   beginning of the C_{H-1} domain, and when cloned into pCD-HC68B at the same site, is in frame with the remaining sequence for the C_{H-1} through C_{H-3} regions of human IgG_1. In the resulting construct, Gλ-1Apcd (Figs. 8A-8F [SEQ ID NO: 13]), the amino acids immediately following the

20   Campath leader are EVQLLE [SEQ ID NO: 17], where the residues LE are encoded by the nucleotide sequence for the XhoI cloning site.

         The light chain of Gλ-1 was PCR amplified from the Gλ-1 phagemid DNA, using primers for the amino terminus

25   and framework 4 of the variable region. The resulting PCR fragment was cut with SacI (site introduced by the amino terminus primer) and AvrII (naturally occurring site in framework 4), and cloned into 43-1pcn at the SacI/AvrII sites. This cloning grafted the variable

30   region of Gλ-1, in frame, onto the constant region of another anti-RSV lambda light chain 43 [P. Tsui et al., J. Immunol., 157: 772-780 (1996)], which had been cloned at SmithKline Beecham from a combinatorial library derived from RNA isolated from human spleen. The SacI

43

site is introduced at the amino terminus by the PCR
primer and, when cloned into 43pcn at the same site, is
preceded in frame by the Campath leader sequence. The
first two amino acids of the mature light chain are

5   therefore deleted. In the resulting construct, Gλ-1Apcn
(Figs. 9A-9E [SEQ ID NO: 14]), the first two amino acids
immediately following the leader are EL, where the
residues EL are encoded by the nucleotide sequence for
the SacI cloning site.

10   The nucleotide sequences of the plasmids Gλ-1Apcd
and Gλ-1Apcn are shown in Figs. 8A-8F [SEQ ID NO: 13]
and 9A-9E [SEQ ID NO: 14] respectively. This set of
vectors was used to produce antibody Gλ-1A in COS cells
and in CHO cells.

15

Example 3: Cloning Of The Corrected Gλ-1 Heavy and Light
Chains

In cloning the variable region of the Gλ-1 heavy
chain from the single chain Fv (scFv) format into the
20   full length format, the fifth amino acid at the amino
terminus was changed from Val to Leu, for cloning
purposes. To correct this change, PCR primers were
designed for the amino terminus of the Gλ-1 heavy chain
cloned into pCD, which reverted the fifth amino acid
25   back to Val. The correction was introduced via the PCR
overlap technique using the correction primers and
primers annealing to sequences within the CMV promoter
and the C_{H-2} constant region as the outside 5' and 3'
primers, respectfully. The final PCR product was
30   digested with restriction enzymes, EcoRI and Bsp120I,
and cloned into the Gλ-1Apcd vector at the same sites to
create Gλ-1Bpcd.

The final construct was sequenced to verify that
the amino terminus of the heavy chain had been corrected
from EVQLLE [SEQ ID NO: 17] to EVQLVE [SEQ ID NO: 18]
(see Fig 6). The nucleotide sequence of coding region

5   for the corrected heavy chain, Gλ-1B, is shown in Figs.
10A-10B [SEQ ID NO: 15].

In cloning the variable region of the Gλ-1 light
chain from the scFv format into the full length format,
changes were introduced at the amino terminus for

10   cloning purposes. Specifically, the first 2 amino acids
(Gln and Ser) of the light chain were deleted and the
third amino acid was changed from Val to Glu. To
correct these changes, PCR primers were designed for the
amino terminus of the Gλ-1 light chain cloned into pCN,

15   which replaced the two deleted amino acids (Gln and Ser)
and reverted the third amino acid back to Val. The
corrections were introduced via the PCR overlap
technique using the correction primers and primers
annealing to sequences within the CMV promoter and the λ

20   constant region as the outside 5' and 3' primers,
respectfully. The final PCR product was digested with
restriction enzymes, EcoRI and AvrII and cloned into the
Gλ-1Apcn vector at the same sites to create Gλ-1Bpcn.

The final construct was sequenced to verify that

25   the amino terminus of the light chain had been corrected
from --EL to QSVL (amino acids 1-4 of SEQ ID NO: 10).

The nucleotide sequence of coding region for the
corrected light chain, Gλ-1B, is shown in Fig. 11 [SEQ
ID NO: 16]. This vector Gλ-1Bpcn, was used with Gλ-

30   1Bpcd to produce antibody Gλ-1B, in COS cells and in CHO
cells.

Example 4:   Production of Gλ-1 mABs in Mammalian Cells

For initial characterization, the mAb constructs for each version, Gλ-1A heavy and light chain, Gλ-1B heavy and light chain, were expressed in COS cells
5   essentially as described in Current Protocols in Molecular Biology, eds F. M. Ausubel *et al*., 1988, John Wiley & Sons, vol. 1, section 9.1. On day 1 after the transfection, the culture growth medium was replaced with a serum-free medium [SmithKline Beecham] which was
10   changed on day 3. Similar satisfactory results are obtained using a publicly available medium, DMEM supplemented with ITS™ Premix, an insulin, transferrin, selenium mixture (Collaborative Research, Bedford, MA) and 1 mg/ml bovine serum albumin (BSA).

15   The mAb was prepared from the day 3 + day 5 conditioned medium by standard protein A affinity chromatography methods (e.g., as described in Protocols in Molecular Biology) using, for example, Prosep A affinity resin (Bioprocessing Ltd., UK).

20   To produce larger quantities of the Gλ-1B mAB (100-200 mgs), the vectors were introduced into a proprietary CHO cell system. However, similar results will be obtained using dhfr⁻ CHO cells as previously described [P. Hensley *et al*., J. Biol. Chem., 269:23949-23958
25   (1994)]. Briefly, a total of 30 μg of linearized plasmid DNA (15 μg each of the A or B set of heavy chain and light chain vectors) is electroporated into 1×10^7 cells. The cells are initially selected in nucleoside-free medium in 96 well plates. After three to four
30   weeks, media from growth positive wells is screened for human immunoglobulin using an ELISA assay. The highest expressing colonies are expanded and selected in increasing concentrations of methotrexate for

amplification of the transfected vectors. The antibody
is purified from conditioned medium by standard
procedures using protein A affinity chromatography
(Protein A sepharose, Pharmacia) followed by size
5   exclusion chromatography (Superdex 200, Pharmacia).

The concentration and the antigen binding activity
of the eluted antibody are measured by ELISA. The
antibody containing fractions are pooled and further
purified by size exclusion chromatography. As expected
10   for any such antibody, by SDS-PAGE, the predominant
protein product migrated at approximately 150 kd under
non-reducing conditions and as two bands of 50 and 25 kd
under reducing conditions. For antibody produced in CHO
cells, the purity was > 90%, as judged by SDS-PAGE, and
15   the concentration was accurately determined by amino
acid analysis.

Example 5:   Binding of the Gλ-1 mABs to recombinant F
protein

20   Binding of the Gλ-1 mABs to recombinant F protein
was measured in a standard solid phase ELISA. Antigen
diluted in PBS pH 7.0 was adsorbed onto polystyrene
round-bottom microplates (Dynatech, Immunolon II) for 18
hours. Wells were then aspirated and blocked with 0.5%
25   boiled casein (BC) in PBS containing 1% Tween 20
(PBS/0.05% BC) for two hours. Antibodies (50 µl/well)
were diluted to varying concentrations in PBS/0.5% BC
containing 0.025% Tween 20 and incubated in antigen
coated wells for one hour. Plates were washed three
30   times with PBS containing 0.05% Tween 20, using a
Titertek 320 microplate washer, followed by addition of
HRP-labelled protein A/G (50 µl) diluted 1:5000. After
washing three times, TMBlue substrate (TSI, #TM102) was
added and plates were incubated an additional 15

47

minutes. The reaction was stopped by addition of 1 N H_2SO_4 and absorbance read at 450 nm using a Biotek ELISA reader.

The antigen binding epitope of the Gλ-1 mABs was

5   examined in a competition ELISA. The Gλ-1 mABs were mixed with increasing concentrations of RSMU19 or B4, two potent neutralizing mAbs [Tempest et al., Biotech., 9: 266-271 (1991); Kennedy et al., J. Gen. Virol., 69: 3023-3032 (1988)] and added to F protein-coated wells.

10   The epitope regions recognized by mAbs RSMU19 and B4 are quite distinct from each other as previously described in Arbiza et al., J. Gen. Virol., 73: 2225-2234 (1992). The concentration of the Gλ-1 mABs used in competition studies was determined previously to give 90% maximal

15   binding to F antigen. Binding of the Gλ-1 mABs in the presence of other mABs was detected using HRP-labelled goat anti-human IgG. The reaction was developed as stated above.

The Gλ-1 mABs demonstrated potent binding to

20   recombinant F (rF) protein by ELISA (EC$_{50}$ for mAB B = 2.6 ng/ml). Binding of the Gλ-1 mABs to rF protein was inhibited by mAb B4, for which the F protein amino acids critical for antigen recognition are amino acids 268, 272 and 275 of SEQ ID NO: 20). Binding of the Gλ-1 mABs

25   to rF protein was not inhibited by mAb RSMU19, for which F protein amino acid 429 of SEQ ID NO: 20 is critical for antigen recognition. These results indicate that residues in the region of amino acids 255-275 of the F protein [SEQ ID NO: 20] are critical for Gλ-1 mAB

30   recognition.

Example 6:   *In vitro Fusion-Inhibition Activity of the*
Gλ-1 mABs

The ability of the Gλ-1 mABs to inhibit virus-
induced cell fusion was determined using a modification
5   of the *in vitro* microneutralization assay [Beeler *et
al.*, J. Virol., 63:2941-2950 (1989)]. In this assay, 50
μl of RS Long strain virus (10-100 TCID$_{50}$/well [American
Type Culture Collection ATCC VR-26] were mixed with 0.1
ml VERO cells (5X10^3/well) [ATCC CCL-81] in Minimum
10   Essential Media (MEM) containing 2% fetal calf serum
(FCS), for 4 hours at 37°C, 5% CO$_2$. Serial two-fold
dilutions (in quadruplicate) of mAB (50 μl) were then
added to wells containing virus-infected cells. Control
cultures contained cells incubated with virus only
15   (positive virus control) or cells incubated with media
alone.

Cultures were incubated at 37°C in 5% CO$_2$ for 6 days
at which time cytopathic effects (CPE) in virus control
wells were > 90%. Microscopic examination for
20   cytopathic effects were confirmed by ELISA. Media was
aspirated from cultures and replaced with 50 μl of 90%
methanol containing 0.6% H$_2$O$_2$. After 10 minutes,
fixative was aspirated and plates were air dried
overnight. Viral antigen was detected in the fixed
25   cultures using 1 μg/ml biotinylated RSCHB4 (a human Fc
derivative of the bovine B4 mAb [SmithKline Beecham]),
followed by HRP-labelled streptavidin (Boehringer-
Mannheim) diluted 1:10,000. The reaction was developed
using TMBlue and stopped by addition of 1N H$_2$SO$_4$.
30   Absorbance was measured at 450 nm (O.D.$_{450}$).

Fusion-inhibition titers were defined as the
concentration of antibody which caused a 50% reduction
in ELISA signal (ED$_{50}$) as compared to virus controls.

Based on the curve generated in the ELISA by the
standard virus titration, a 50% reduction in $O.D._{450}$
corresponded to $\geq$ 90% reduction in virus titer.
Calculation of the 50% point was based on regression
5   analysis of the dose titration.

The Gλ-1 mABs demonstrated potent *in vitro* fusion-
inhibition activity against type A RS Long strain virus
(ED_{50} for mAB B of 0.51 ± 0.38 μg/ml). In this *in vitro*
fusion-inhibition assay, Gλ-1 mAB B was more active than
10   the humanized mAB RSHZ19 (ED_{50} of 0.4-3.0 μg/ml) [Wyde *et
al*., Pediatr. Res., 38(4):543-550] in comparative
assays.

Example 7:   *In vivo* Activity of Gλ-1 mAB B: Prophylaxis
15   and Therapy in Balb/c Mouse Model
Balb/c mice (5/group) were inoculated
intraperitoneally with doses ranging from 0.06 mg/kg to
5 mg/kg of Gλ-1 mAB B either 24 hours prior
(prophylaxis) or 4 days after (therapy) intranasal
20   infection with 10^5 PFU of the A2 strain of human RSV.
Mice were sacrificed 5 days after infection. Lungs were
harvested and homogenized to determine virus titers.
Virus was undetectable in the lungs of mice treated
prophylactically with $\geq$ 1.25 mg/kg Gλ-1 mAB B either
25   prophylactically or therapeutically. See Table II
below. Significant viral clearance (2-3 log_{10}) was also
achieved in animals receiving 0.31 mg/kg Gλ-1 mAB B
either prophylactically or therapeutically.

30

Table II: Gλ-1 mAB B Prophylaxis and Therapy in Balb/c Mice

Treatment	Dose (mg/kg)	Lung Virus Titer ($\log_{10}$/g lung) Prophylaxis	Therapy
Gλ-1 mAB B	5	<1.7	<1.7
	1.25	<1.7	<1.7
	0.31	1.8 ± 0.3	2.9 ± 0.4
	0.06	4.3 ± 0.7	4.5 ± 0.3
PBS	-	4.8 ± 0.7	4.7 ± 0.2

The Gλ-1 mABs have potent antiviral activity *in vitro* against a broad range of native RSV isolates of both type A and B, and show prophylactic and therapeutic efficacy *in vivo* in animal models. Thus, the Gλ-1 mABs are candidates for therapeutic, prophylactic, and diagnostic application in man.

Numerous modifications and variations of the present invention may be made by one of skill in the art in view of the invention described herein. Such modifications are believed to be encompassed by the specification and claims of the present invention. All references cited above are incorporated by reference herein.

WHAT IS CLAIMED IS:

1.   A human monoclonal antibody and
functional fragments thereof, specifically reactive with
an F protein epitope of Respiratory Syncytial Virus and
capable of neutralizing infection by said virus selected
from the group consisting of Gλ-1A and Gλ-1B.

2.   The monoclonal antibody according to
Claim 1 which comprises the light chain amino acid
sequence of Fig. 3 SEQ ID NO: 2 and the heavy chain
amino acid sequence of Fig. 4 SEQ ID NO: 4.

3.   The monoclonal antibody according to
Claim 1 which comprises the light chain amino acid
sequence encoded by the DNA sequence of Fig. 11 SEQ ID
NO: 16 and the heavy chain amino acid sequence encoded
by the DNA sequence of Figs. 10A-10B SEQ ID NO: 15.

4.   The monoclonal antibody according to
Claim 1 wherein said fragment is selected from the group
consisting of Fv, Fab and F(ab')$_2$.

5.   An isolated nucleic acid molecule
selected from the group consisting of:
        (a) a nucleic acid sequence encoding any
of the human monoclonal antibodies, altered antibodies
and CDRs of any of the claims 1-4;
        (b) a nucleic acid complementary to any
of the sequences in (a); and
        (c) a nucleic acid sequence of 18 or more
nucleotides capable of hybridizing to the CDRs of any of
claims 1-4 under stringent conditions.

6.   The isolated nucleic acid molecule according to Claim 5 comprising the sequences of Figs. 8A-8F and 9A-9E SEQ ID NOS: 13 and 14, or Figs. 10A-10B and 11 SEQ ID NOS: 15 and 16.

7.   A recombinant plasmid comprising the nucleic acid sequences of any of Claims 5 or 6.

8.   A host cell comprising the plasmid of Claim 7.

9.   A process for the production of a human antibody specific for RSV comprising culturing the host cell of Claim 8 in a medium under suitable conditions of time temperature and pH and recovering the antibody so produced.

10.   A method of detecting RSV comprising contacting a source suspected of containing RSV with a diagnostically effective amount of the monoclonal antibody of Claim 1 and determining whether the monoclonal antibody binds to the source.

11.   A method for providing passive immunotherapy to RSV disease in a human, comprising administering to the human an immunotherapeutically effective amount of the monoclonal antibody of Claim 1.

12.   The method according to Claim 11 wherein the passive immunotherapy is provided prophylactically.

13.   A pharmaceutical composition comprising at least one dose of an immunotherapeutically effective

amount of the monoclonal antibody of Claim 1 in a
pharmaceutically acceptable carrier.

14. A pharmaceutical composition comprising
at least one dose of an immunotherapeutically effective
amount of the monoclonal antibody of Claim 1 in
combination with at least one additional monoclonal
antibody.

15. The pharmaceutical composition according
to Claim 14 wherein said additional monoclonal antibody
is an anti-RSV antibody distinguished from the antibody
of Claim 1 by virtue of being reactive with a different
epitope of the RSV F protein antigen.

Fig. 1A

RSV19/GI1 scFv phage competition
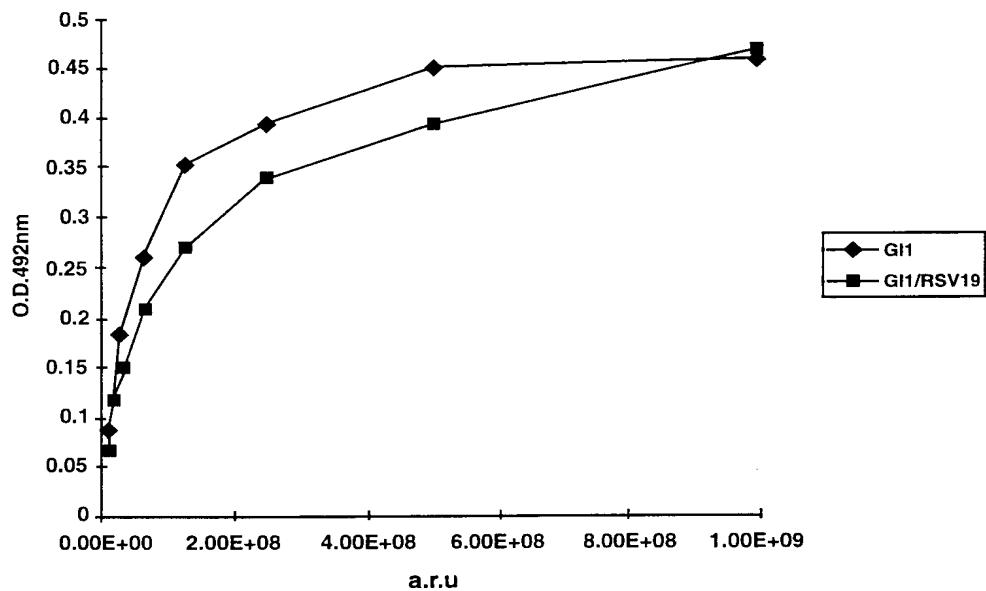
Fig. 1B

B4/GI1 scFv phage competition
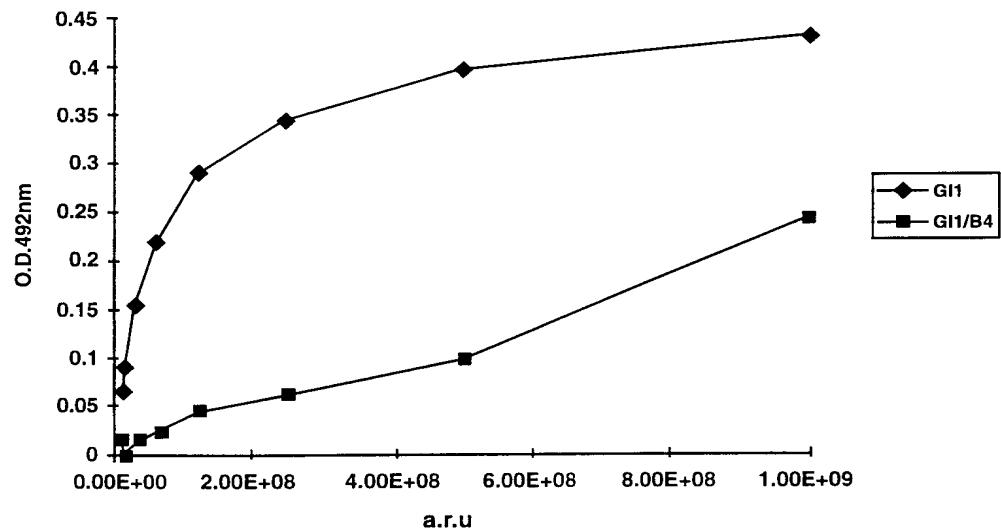
Fig. 2

Neutralisation of RS/V/273 with phage Fv
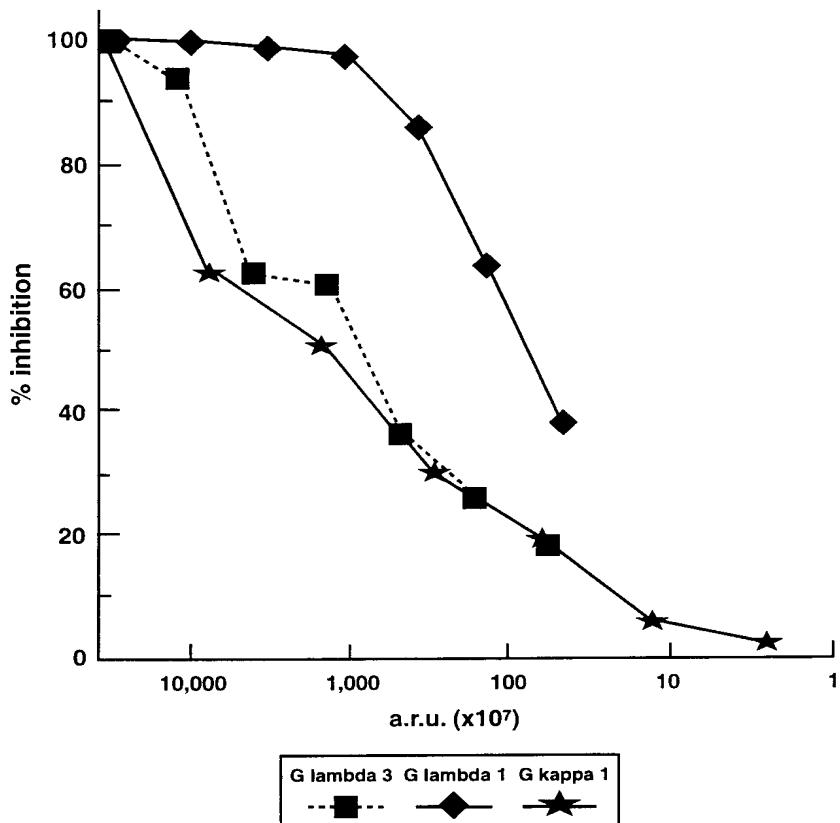
FIGURE 3

```
  1   CAGTCTGTGTTGACGCAGCCGCCCTCAGTCTCTGCGGCCCCAGGACAGAA     50
      Q   S   V   L   T   Q   P   P   S   V   S   A   A   P   G   Q   K

 51   GGTCACCATCTCCTGCACTGGGAGCAGCTCCAACCTCGGGGCAGGTTATG    100
       V   T   I   S   C   T   G   S   S   S   N   L   G   A   G   Y   D

101   ATGTTCACTGGTACCGGCAACTTCCAGGGACAGCCCCCAAACTCCTCATC    150
        V   H   W   Y   R   Q   L   P   G   T   A   P   K   L   L   I

151   TATGATAACAACAATCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTC    200
        Y   D   N   N   N   R   P   S   G   V   P   D   R   F   S   G   S

201   CAAGTCTGGCCCCTCAGCCTCCCTGGCCATCTCTGGGCTCCAGGCTGAGG    250
        K   S   G   P   S   A   S   L   A   I   S   G   L   Q   A   E   D

251   ATGAGGCTGATTATTACTGCCAGTCCTATGACAGCAGCCTGAATGGTTAT    300
        E   A   D   Y   Y   C   Q   S   Y   D   S   S   L   N   G   Y

301   GTCTTCGGAACTGGGACCCAGCTCACCGTCCTAGGT                 336
        V   F   G   T   G   T   Q   L   T   V   L   G
```

FIGURE 4

```
  1    GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTC      50
       E   V   Q   L   V   E   S   G   G   G   L   V   Q   P   G   G   S


 51    CCTGAGACTCTCCTGCGCAGCCTCTGGAGTCTCCCTCAGTGGATACAAGA     100
        L   R   L   S   C   A   A   S   G   V   S   L   S   G   Y   K   M


101    TGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGGTCTCTTCC     150
         N   W   V   R   Q   A   P   G   K   G   L   E   W   V   S   S


151    ATTACTGGTATGAGTAATTACATACACTACTCAGACTCAGTGAAGGGCCG     200
        I   T   G   M   S   N   Y   I   H   Y   S   D   S   V   K   G   R


201    ATTCACCATCTCCAGAGACAACGCCATGAACTCACTGTATCTGCAAATGA     250
         F   T   I   S   R   D   N   A   M   N   S   L   Y   L   Q   M   N


251    ACAGCCTGACAGCCGAGGACACGGGTGTTTATTATTGTGCGACACAACCG     300
         S   L   T   A   E   D   T   G   V   Y   Y   C   A   T   Q   P


301    GGGGAGCTGGCGCCTTTTGACCATTGGGGCCAGGGAACCCTGGTCACCGT     350
        G   E   L   A   P   F   D   H   W   G   Q   G   T   L   V   T   V


351    CTCCTCA                                                357
        S   S
```

Figure 5
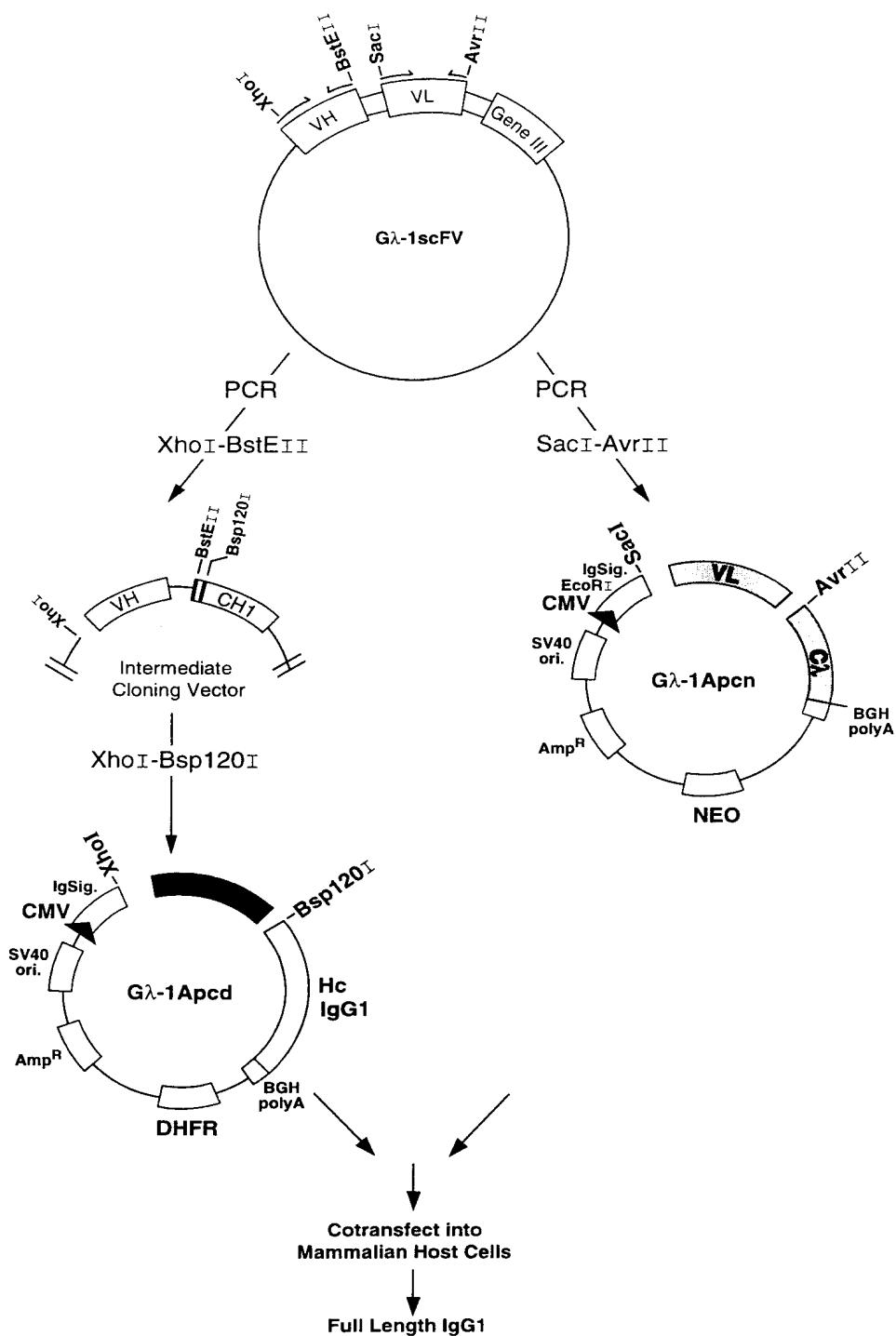
FIGURE 6

**Comparison of the Heavy Chain Amino Acid Sequences of the
Gλ-1 single chain fv and mAbs**

Leader and Variable Regions


```
GL Dp58:                              EVQLVESGGGLVQPGGSLRLSCAASGFTFS
Gλ-1 scFv:                            ------------------------------VSL-
Gλ-1A:         MGWSCIILFLVATATGVHS----L--------------------------
Gλ-1B:         --------------------V--------------------------
```

```
               CDR1                       CDR2
               -----              -----------------
GL Dp58:       SYEMNWVRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLY
Gλ-1 scFV:     G-K---------------S-TGMSNY-H-S-------------M----
Gλ-1A:         ---------------------------------------------------
Gλ-1B:         -----------------------------------------------
```

```
                                CDR3
                            ---------
GL: Dp58:      LQMNSLRAEDTAVYYCAR
Gλ-1 scFv:     ------T----G-----TQPGELAPFDHWGQGTLVTVSS
Gλ-1A:         ----------------------------------------
Gλ-1B:         ----------------------------------------
```

FIG 7

**Comparison of the Light Chain Amino Acid Sequences of the Gλ-1A:
single chain Fv and mAbs**

Leader and Variable Regions

```
                                                              CDR1
                                                            --------
GL DpL8:                          QSVLTQPPSVSGAPGQRVTISCTGSSSNIG
Gλ-1 scFv:                        -----------A----K----------L-
Gλ-1A:      MGWSCIILFLVATATGVHS   E---------------------------
Gλ-1B:      -------------------QSV------------------------
```

```
                              CDR2
            ------                    -------
GL DpL8:    AGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGL
Gλ-1scFv:   --------R-----------D-N--------------P------S--
Gλ-1A:      ------------------------------------------------
Gλ-1B:      ------------------------------------------------
```

```
                        CDR3
            -----------
GL DpL8:    QAEDEADYYC
Gλ-1 scFv:  ----------QSYDSSLNGYVFGTGTQLTVLG
Gλ-1A:      -------------------------------
Gλ-1B:      -------------------------------
```

FIGURE 8A

```
   1  gacgtcgcggccgctctaggcctccaaaaaagcctcctcactacttctgg

  51  aatagctcagaggccgaggcggcctcggcctctgcataaataaaaaaaat

 101  tagtcagccatgcatggggcggagaatgggcggaactgggcggagttagg

 151  ggcgggatgggcggagttaggggcgggactatggttgctgactaattgag

 201  atgcatgctttgcatacttctgcctgctggggagcctggggactttccac

 251  acctggttgctgactaattgagatgcatgctttgcatacttctgcctgct

 301  ggggagcctggggactttccacaccctaactgacacacattccacagaat

 351  taattcccggggatcgatccgtcgacgtacgactagttattaatagtaat

 401  caattacggggtcattagttcatagcccatatatggagttccgcgttaca

 451  taacttacggtaaatggcccgcctggctgaccgcccaacgacccccgccc

 501  attgacgtcaataatgacgtatgttcccatagtaacgccaatagggactt

 551  tccattgacgtcaatgggtggactatttacggtaaactgcccacttggca

 601  gtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatga

 651  cggtaaatggcccgcctggcattatgcccagtacatgaccttatgggact

 701  ttcctacttggcagtacatctacgtattagtcatcgctattaccatggtg

 751  atgcggttttggcagtacatcaatgggcgtggatagcggtttgactcacg

 801  gggatttccaagtctccacccattgacgtcaatgggagtttgttttggc

 851  accaaaatcaacgggactttccaaaatgtcgtaacaactccgccccattg

 901  acgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagc
```

<div align="right">EcoRI</div>

```
 951  tgggtacgtgaaccgtcagatcgcctggagacgccatcgaattctgagca
```

```
1001  cacaggacctcaccatgggatggagctgtatcatcctcttcttggtagca
                           M  G  W  S  C  I  I  L  F  L  V  A
                          Leader start
```

<div align="right">XhoI</div>

```
1051  acagctacaggtgtccactccgaggtccaactgctcgagtctggggggagg
        T  A  T  G  V  H  S  E  V  Q  L  L  E  S---
                                   Processed N-term
```

FIGURE 8B

1101   cttggtacagcctggggggggtccctgagactctcctgcgcagcctctggag

1151   tctccctcagtggatacaagatgaactgggtccgccaggctccagggaag

1201   gggctggaatgggtctcttccattactggtatgagtaattacatacacta

1251   ctcagactcagtgaagggccgattcaccatctccagagacaacgccatga

1301   actcactgtatctgcaaatgaacagcctgacagccgaggacacgggtgtt

1351   tattattgtgcgacacaaccgggggagctggcgccttttgaccattgggg

```
                BstEII                              Bsp120I
1401    ccagggaaccctggtcaccgtctcctcagcctccaccaagggcccatcgg
        Q   G   T   L   V   T   V   S   S   /
                            framework IV / CH1
```

1451   tcttccccctggcaccctcctccaagagcacctctgggggcacagcggcc

1501   ctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtg

1551   gaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctac

```
                                        BstEII
1601    agtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagc
```

1651   agcttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaa

1701   caccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcaca

1751   catgcccaccgtgcccagcacctgaactcctggggggaccgtcagtcttc

1801   ctcttccccccaaaacccaaggacaccctcatgatctcccggacccctga

1851   ggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagt

1901   tcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccg

1951   cgggaggagcagtacaacagcacgtaccgggtggtcagcgtcctcaccgt

2001   cctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctcca

2051   acaaagccctcccagcccccatcgagaaaaccatctccaaagccaaaggg

2101   cagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagct

2151   gaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccca

FIGURE 8C

```
2201   gcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactac

2251   aagaccacgcctcccgtgctggactccgacggctccttcttcctctacag

2301   caagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcat

2351   gctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc

2401   tccctgtctccgggtaaatgatagatatctacgtatgatcagcctcgact
              S   P   G   K   *   C-term of heavy chain

2451   gtgccttctagttgccagccatctgttgtttgcccctcccccgtgccttc

2501   cttgaccctggaaggtgccactcccactgtcctttcctaataaaatgagg

2551   aaattgcatcgcattgtctgagtaggtgtcattctattctggggggtggg

2601   gtggggcaggacagcaaggggggaggattgggaagacaatagcaggcatgc

2651   tggggatgcggtgggctctatggaaccagctggggctcgacagcgctgga

2701   tctcccgatccccagctttgcttctcaatttcttatttgcataatgagaa

2751   aaaaggaaaattaattttaacaccaattcagtagttgattgagcaaatg

2801   cgttgccaaaaaggatgctttagagacagtgttctctgcacagataagga

2851   caaacattattcagagggagtacccagagctgagactcctaagccagtga

2901   gtggcacagcattctaggagaaatatgcttgtcatcaccgaagcctgat

2951   tccgtagagccacaccttggtaagggccaatctgctcacacaggatagag

3001   agggcaggagccagggcagagcatataaggtgaggtaggatcagttgctc

3051   ctcacatttgcttctgacatagttgtgttgggagcttggatagcttggac

3101   agctcagggctgcgatttcgcgccaaacttgacggcaatcctagcgtgaa

3151   ggctggtaggattttatccccgctgccatcatggttcgaccattgaactg

3201   catcgtcgccgtgtcccaaaatatggggattggcaagaacggagacctac

3251   cctggcctccgctcaggaacgagttcaagtacttccaaagaatgaccaca

3301   acctcttcagtggaaggtaaacagaatctggtgattatgggtaggaaaac

3351   ctggttctccattcctgagaagaatcgacctttaaaggacagaattaata
```

FIGURE 8D

3401 tagttctcagtagagaactcaaagaaccaccacgaggagctcattttctt

3451 gccaaaagtttggatgatgccttaagacttattgaacaaccggaattggc

3501 aagtaaagtagacatggtttggatagtcggaggcagttctgtttaccagg

3551 aagccatgaatcaaccaggccaccttagactctttgtgacaaggatcatg

3601 caggaatttgaaagtgacacgttttcccagaaattgatttggggaaata

3651 taaacttctcccagaatacccaggcgtcctctctgaggtccaggaggaaa

3701 aaggcatcaagtataagtttgaagtctacgagaagaaagactaacaggaa

3751 gatgctttcaagttctctgctccctcctaaagctatgcatttttataag

3801 accatgggacttttgctggctttagatcagcctcgactgtgccttctagt

3851 tgccagccatctgttgtttgccctcccccgtgccttccttgaccctgga

3901 aggtgccactcccactgtcctttcctaataaaatgaggaaattgcatcgc

3951 attgtctgagtaggtgtcattctattctgggggtgggtggggcaggac

4001 agcaaggggaggattgggaagacaatagcaggcatgctggggatgcggt

4051 gggctctatggaaccagctggggctcgatcgagtgtatgactgcggccgc

4101 gatcccgtcgagagcttggcgtaatcatggtcatagctgtttcctgtgtg

4151 aaattgttatccgctcacaattccacacaacatacgagccggaagcataa

4201 agtgtaaagcctggggtgcctaatgagtgagctaactcacattaattgcg

4251 ttgcgctcactgcccgctttccagtcgggaaacctgtcgtgccagctgca

4301 ttaatgaatcggccaacgcgcggggagaggcggtttgcgtattgggcgct

4351 cttccgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcgg

4401 cgagcggtatcagctcactcaaaggcggtaatacggttatccacagaatc

4451 aggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggcca

4501 ggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgcccc

4551 cctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaaccc

4601 gacaggactataaagataccaggcgtttcccctggaagctccctcgtgc

FIGURE 8E

```
4651   gctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctc

4701   ccttcgggaagcgtggcgctttctcaatgctcacgctgtaggtatctcag

4751   ttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaacccccccg

4801   ttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaac

4851   ccggtaagacacgacttatcgccactggcagcagccactggtaacaggat

4901   tagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggc

4951   ctaactacggctacactagaaggacagtatttggtatctgcgctctgctg

5001   aagccagttaccttcggaaaaagagttggtagctcttgatccggcaaaca

5051   aaccaccgctggtagcggtggtttttttgtttgcaagcagcagattacgc

5101   gcagaaaaaaaggatctcaagaagatcctttgatcttttctacggggtct

5151   gacgctcagtggaacgaaaactcacgttaagggattttggtcatgagatt

5201   atcaaaaaggatcttcacctagatccttttaaattaaaaatgaagtttta

5251   aatcaatctaaagtatatatgagtaaacttggtctgacagttaccaatgc

5301   ttaatcagtgaggcacctatctcagcgatctgtctatttcgttcatccat

5351   agttgcctgactccccgtcgtgtagataactacgatacgggagggcttac

5401   catctggccccagtgctgcaatgataccgcgagacccacgctcaccggct

5451   ccagatttatcagcaataaaccagccagccggaagggccgagcgcagaag

5501   tggtcctgcaactttatccgcctccatccagtctattaattgttgccggg

5551   aagctagagtaagtagttcgccagttaatagtttgcgcaacgttgttgcc

5601   attgctacaggcatcgtggtgtcacgctcgtcgtttggtatggcttcatt

5651   cagctccggttcccaacgatcaaggcgagttacatgatcccccatgttgt

5701   gcaaaaaagcggttagctccttcggtcctccgatcgttgtcagaagtaag

5751   ttggccgcagtgttatcactcatggttatggcagcactgcataattctct

5801   tactgtcatgccatccgtaagatgcttttctgtgactggtgagtactcaa
```

FIGURE 8F

```
5851   ccaagtcattctgagaatagtgtatgcggcgaccgagttgctcttgcccg

5901   gcgtcaatacgggataataccgcgccacatagcagaactttaaaagtgct

5951   catcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgc

6001   tgttgagatccagttcgatgtaacccactcgtgcacccaactgatcttca

6051   gcatcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggca

6101   aaatgccgcaaaaaagggaataagggcgacacggaaatgttgaatactca

6151   tactcttcctttttcaatattattgaagcatttatcagggttattgtctc

6201   atgagcggatacatatttgaatgtatttagaaaaataaacaaatagggggt

6251   tccgcgcacatttccccgaaaagtgccacct
```

FIGURE 9A

```
   1   gacgtcgcggccgctctaggcctccaaaaaagcctcctcactacttctgg

  51   aatagctcagaggccgaggcggcctcggcctctgcataaataaaaaaaat

 101   tagtcagccatgcatggggcggagaatgggcggaactgggcggagttagg

 151   ggcgggatgggcggagttaggggcgggactatggttgctgactaattgag

 201   atgcatgctttgcatacttctgcctgctggggagcctggggactttccac

 251   acctggttgctgactaattgagatgcatgctttgcatacttctgcctgct

 301   ggggagcctggggactttccacaccctaactgacacacattccacagaat

 351   taattcccggggatcgatccgtcgacgtacgactagttattaatagtaat

 401   caattacggggtcattagttcatagcccatatatggagttccgcgttaca

 451   taacttacggtaaatggcccgcctggctgaccgcccaacgacccccgccc

 501   attgacgtcaataatgacgtatgttcccatagtaacgccaatagggactt

 551   tccattgacgtcaatgggtggactatttacggtaaactgcccacttggca

 601   gtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatga

 651   cggtaaatggcccgcctggcattatgcccagtacatgaccttatgggact

 701   ttcctacttggcagtacatctacgtattagtcatcgctattaccatggtg

 751   atgcggttttggcagtacatcaatgggcgtggatagcggtttgactcacg

 801   gggatttccaagtctccacccattgacgtcaatgggagtttgttttggc

 851   accaaaatcaacgggactttccaaaatgtcgtaacaactccgccccattg

 901   acgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagc
```

```
                                                          EcoRI
 951   tgggtacgtgaaccgtcagatcgcctggagacgccatcgaattctgagca

1001   cacaggacctcaccatgggatggagctgtatcatcctcttcttggtagca
                        M   G   W   S   C   I   I   L   F   L   V   A
                        Leader start


                       SacI
1051   acagctacaggtgtccactccgagctcacgcagccgccctcagtctctgc
        T   A   T   G   V   H   S   E   L   T   Q  --
                                        Processed N-term
```

FIGURE 9B

```
1101   ggccccaggacagaaggtcaccatctcctgcactgggagcagctccaacc

1151   tcggggcaggttatgatgttcactggtaccggcaacttccagggacagcc

1201   cccaaactcctcatctatgataacaacaatcggccctcaggggtccctga

1251   ccgattctctggctccaagtctggcccctcagcctccctggccatctctg

1301   ggctccaggctgaggatgaggctgattattactgccagtcctatgacagc
```

<pre>
                                                   AvrII
1351   agcctgaatggttatgtcttcggaactgggacccagctcaccgtcctagg
                                T   Q   L   T   V   L   G
                                Framework IV / Cλ
</pre>

```
1401   tcagcccaaggctgcccctcggtcactctgttcccgccctcctctgagg

1451   agcttcaagccaacaaggccacactggtgtgtctcataagtgacttctac

1501   ccgggagccgtgacagtggcctggaaggcaattagcagccccgtcaaggc

1551   gggagtggagaccaccacaccctccaaacaaagcaacaacaagtacgcgg

1601   ccagcagctatctgagcctgacgcctgagcagtggaagtcccacagaagg

1651   tacagctgccaggtcacgcatgaagggagcaccgtggagaagacagtggc
```

<pre>
1701   ccctacagaatgttcatagttctagatctacgtatgatcagcctcgactg
          P   T   E   C   S   * C-term light chain
</pre>

```
1751   tgccttctagttgccagccatctgttgtttgcccctcccccgtgccttcc

1801   ttgaccctggaaggtgccactcccactgtcctttcctaataaaatgagga

1851   aattgcatcgcattgtctgagtaggtgtcattctattctggggggtgggg

1901   tggggcaggacagcaaggggggaggattgggaagacaatagcaggcatgct

1951   ggggatgcggtgggctctatggaaccagctggggctcgacagctcgagct

2001   agctttgcttctcaatttcttatttgcataatgagaaaaaaaggaaaatt

2051   aattttaacaccaattcagtagttgattgagcaaatgcgttgccaaaaag

2101   gatgctttagagacagtgttctctgcacagataaggacaaacattattca

2151   gagggagtacccagagctgagactcctaagccagtgagtggcacagcatt
```

FIGURE 9C

```
2201  ctagggagaaatatgcttgtcatcaccgaagcctgattccgtagagccac

2251  accttggtaagggccaatctgctcacacaggatagagagggcaggagcca

2301  gggcagagcatataaggtgaggtaggatcagttgctcctcacatttgctt

2351  ctgacatagttgtgttgggagcttggatcgatccaccatggttgaacaag

2401  atggattgcacgcaggttctccggccgcttgggtggagaggctattcggc

2451  tatgactgggcacaacagacaatcggctgctctgatgccgccgtgttccg

2501  gctgtcagcgcaggggcgcccggttcttttgtcaagaccgacctgtccg

2551  gtgccctgaatgaactgcaggacgaggcagcgcggctatcgtggctggcc

2601  acgacgggcgttccttgcgcagctgtgctcgacgttgtcactgaagcggg

2651  aagggactggctgctattgggcgaagtgccggggcaggatctcctgtcat

2701  ctcaccttgctcctgccgagaaagtatccatcatggctgatgcaatgcgg

2751  cggctgcatacgcttgatccggctacctgcccattcgaccaccaagcgaa

2801  acatcgcatcgagcgagcacgtactcggatggaagccggtcttgtcgatc

2851  aggatgatctggacgaagagcatcaggggctcgcgccagccgaactgttc

2901  gccaggctcaaggcgcgcatgcccgacggcgaggatctcgtcgtgaccca

2951  tggcgatgcctgcttgccgaatatcatggtggaaaatggccgcttttctg

3001  gattcatcgactgtggccggctgggtgtggcggaccgctatcaggacata

3051  gcgttggctaccgtgatattgctgaagagcttggcggcgaatgggctga

3101  ccgcttcctcgtgctttacggtatcgccgctcccgattcgcagcgcatcg

3151  ccttctatcgccttcttgacgagttcttctgagcgggactctggggttcg

3201  aaatgaccgaccaagcgacgcccaacctgccatcacgagatttcgattcc

3251  accgccgccttctatgaaaggttgggcttcggaatcgttttccgggacgc

3301  cggctggatgatcctccagcgcggggatctcatgctggagttcttcgccc

3351  accccaacttgtttattgcagcttataatggttacaaataaagcaatagc
```

FIGURE 9D

3401   atcacaaatttcacaaataaagcattttttttcactgcattctagttgtgg

3451   tttgtccaaactcatcaatgtatcttatcatgtctggatcgcggccgcga

3501   tcccgtcgagagcttggcgtaatcatggtcatagctgtttcctgtgtgaa

3551   attgttatccgctcacaattccacacaacatacgagccggaagcataaag

3601   tgtaaagcctggggtgcctaatgagtgagctaactcacattaattgcgtt

3651   gcgctcactgcccgctttccagtcgggaaacctgtcgtgccagctgcatt

3701   aatgaatcggccaacgcgcggggagaggcggtttgcgtattgggcgctct

3751   tccgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggcg

3801   agcggtatcagctcactcaaaggcggtaatacggttatccacagaatcag

3851   gggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccagg

3901   aaccgtaaaaaggccgcgttgctggcgtttttccataggctccgcccccc

3951   tgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccga

4001   caggactataaagataccaggcgtttccccctggaagctccctcgtgcgc

4051   tctcctgttccgaccctgccgcttaccggatacctgtccgcctttctccc

4101   ttcgggaagcgtggcgctttctcaatgctcacgctgtaggtatctcagtt

4151   cggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgtt

4201   cagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaaccc

4251   ggtaagacacgacttatcgccactggcagcagccactggtaacaggatta

4301   gcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggcct

4351   aactacggctacactagaaggacagtatttggtatctgcgctctgctgaa

4401   gccagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaa

4451   ccaccgctggtagcggtggtttttttgtttgcaagcagcagattacgcgc

4501   agaaaaaaaggatctcaagaagatcctttgatcttttctacggggtctga

4551   cgctcagtggaacgaaaactcacgttaagggattttggtcatgagattat

FIGURE 9E

4601   caaaaaggatcttcacctagatccttttaaattaaaaatgaagttttaaa

4651   tcaatctaaagtatatatgagtaaacttggtctgacagttaccaatgctt

4701   aatcagtgaggcacctatctcagcgatctgtctatttcgttcatccatag

4751   ttgcctgactccccgtcgtgtagataactacgatacgggagggcttacca

4801   tctggccccagtgctgcaatgataccgcgagacccacgctcaccggctcc

4851   agatttatcagcaataaaccagccagccggaagggccgagcgcagaagtg

4901   gtcctgcaactttatccgcctccatccagtctattaattgttgccgggaa

4951   gctagagtaagtagttcgccagttaatagtttgcgcaacgttgttgccat

5001   tgctacaggcatcgtggtgtcacgctcgtcgtttggtatggcttcattca

5051   gctccggttcccaacgatcaaggcgagttacatgatcccccatgttgtgc

5101   aaaaaagcggttagctccttcggtcctccgatcgttgtcagaagtaagtt

5151   ggccgcagtgttatcactcatggttatggcagcactgcataattctctta

5201   ctgtcatgccatccgtaagatgcttttctgtgactggtgagtactcaacc

5251   aagtcattctgagaatagtgtatgcggcgaccgagttgctcttgcccggc

5301   gtcaatacgggataataccgcgccacatagcagaactttaaaagtgctca

5351   tcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgctg

5401   ttgagatccagttcgatgtaacccactcgtgcacccaactgatcttcagc

5451   atcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaa

5501   atgccgcaaaaaagggaataagggcgacacggaaatgttgaatactcata

5551   ctcttcctttttcaatattattgaagcatttatcagggttattgtctcat

5601   gagcggatacatatttgaatgtatttagaaaaataaacaaataggggttc

5651   cgcgcacatttccccgaaaagtgccacct

FIGURE 10A

```
                                              EcoRI
                                              gaattctgagca     1000

cacaggacctcaccatgggatggagctgtatcatcctcttcttggtagca            1050
              M  G  W  S  C  I  I  L  F  L  V  A

acagctacaggtgtccactccgaggtgcagctggtggagtctggggggagg           1100
 T  A  T  G  V  H  S  E  V  Q  L  V  E  S  -
                            N-term

cttggtacagcctggggggtccctgagactctcctgcgcagcctctggag           1150

tctccctcagtggatacaagatgaactgggtccgccaggctccagggaag           1200

gggctggaatgggtctcttccattactggtatgagtaattacatacacta           1250

ctcagactcagtgaagggccgattcaccatctccagagacaacgccatga           1300

actcactgtatctgcaaatgaacagcctgacagccgaggacacgggtgtt           1350

tattattgtgcgacacaaccggggggagctggcgccttttgaccattgggg           1400

                                              Bsp120I
ccagggaaccctggtcaccgtctcctcagcctccaccaagggcccatcgg           1450

tcttcccctggcaccctcctccaagagcacctctgggggcacagcggcc           1500

ctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtg           1550

gaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctac           1600

agtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagc           1650

agcttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaa           1700

caccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcaca           1750

catgcccaccgtgcccagcacctgaactcctggggggaccgtcagtcttc           1800

ctcttccccccaaaacccaaggacaccctcatgatctcccggacccctga           1850

ggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagt           1900

tcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccg           1950

cgggaggagcagtacaacagcacgtaccgggtggtcagcgtcctcaccgt           2000

cctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctcca           2050
```

FIGURE 10B

acaaagccctcccagcccccatcgagaaaaccatctccaaagccaaaggg   2100

cagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagct   2150

gaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccca   2200

gcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactac   2250

aagaccacgcctcccgtgctggactccgacggctccttcttcctctacag   2300

caagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcat   2350

gctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc   2400

tccctgtctccgggtaaatgatagatatct
          S   P   G   K   *

FIGURE 11

```
                                          EcoRI
                                          gaattctgagca              1000

cacaggacctcaccatgggatggagctgtatcatcctcttcttggtagca                 1050
             M   G   W   S   C   I   I   L   F   L   V   A

acagctacaggtgtccactcccagtctgtgttgacgcagccgccctcagt                 1100
 T   A   T   G   V   H   S   Q   S   V   L   T   Q   -
                             N-term

ctctgcggccccaggacagaaggtcaccatctcctgcactgggagcagct                 1150

ccaacctcggggcaggttatgatgttcactggtaccggcaacttccaggg                 1200

acagcccccaaactcctcatctatgataacaacaatcggccctcagggt                  1250

ccctgaccgattctctggctccaagtctggcccctcagcctccctggcca                 1300

tctctgggctccaggctgaggatgaggctgattattactgccagtcctat                 1350

gacagcagcctgaatggttatgtcttcggaactgggacccagctcaccgt                 1400

AvrII
cctaggtcagcccaaggctgcccctcggtcactctgttcccgccctcct                  1450

ctgaggagcttcaagccaacaaggccacactggtgtgtctcataagtgac                 1500

ttctacccgggagccgtgacagtggcctggaaggcaattagcagccccgt                 1550

caaggcgggagtggagaccaccacaccctccaaacaaagcaacaacaagt                 1600

acgcggccagcagctatctgagcctgacgcctgagcagtggaagtcccac                 1650

agaaggtacagctgccaggtcacgcatgaagggagcaccgtggagaagac                 1700

agtggcccctacagaatgttcatagttctagatctacgtatgatcagcct                 1750
        P   T   E   C   S   *
```

SEQUENCE LISTING

(1) GENERAL INFORMATION:

   (i) APPLICANT: SmithKline Beecham, PLC

  (ii) TITLE OF INVENTION: Human Monoclonal Antibody

 (iii) NUMBER OF SEQUENCES: 21

  (iv) CORRESPONDENCE ADDRESS:
     (A) ADDRESSEE: SmithKline Beecham Corporation
     (B) STREET: 709 Swedeland Road
     (C) CITY: King of Prussia
     (D) STATE: PA
     (E) COUNTRY: USA
     (F) ZIP: 19406-2799

   (v) COMPUTER READABLE FORM:
     (A) MEDIUM TYPE: Floppy disk
     (B) COMPUTER: IBM PC compatible
     (C) OPERATING SYSTEM: PC-DOS/MS-DOS
     (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

  (vi) CURRENT APPLICATION DATA:
     (A) APPLICATION NUMBER: GB
     (B) FILING DATE:
     (C) CLASSIFICATION:

 (viii) ATTORNEY/AGENT INFORMATION:
     (A) NAME: King, William T.
     (B) REGISTRATION NUMBER: 30,954
     (C) REFERENCE/DOCKET NUMBER: #

  (ix) TELECOMMUNICATION INFORMATION:
     (A) TELEPHONE: 610-270-4800
     (B) TELEFAX: 610-270-4026

(2) INFORMATION FOR SEQ ID NO:1:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 336 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: double
     (D) TOPOLOGY: unknown

  (ii) MOLECULE TYPE: cDNA

  (ix) FEATURE:
     (A) NAME/KEY: CDS
     (B) LOCATION: 1..336

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CAG TCT GTG TTG ACG CAG CCG CCC TCA GTC TCT GCG GCC CCA GGA CAG     48
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1               5                  10                  15

AAG GTC ACC ATC TCC TGC ACT GGG AGC AGC TCC AAC CTC GGG GCA GGT     96
```

Lys Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Leu Gly Ala Gly
              20   25   30

TAT GAT GTT CAC TGG TAC CGG CAA CTT CCA GGG ACA GCC CCC AAA CTC   144
Tyr Asp Val His Trp Tyr Arg Gln Leu Pro Gly Thr Ala Pro Lys Leu
          35   40   45

CTC ATC TAT GAT AAC AAC AAT CGG CCC TCA GGG GTC CCT GAC CGA TTC   192
Leu Ile Tyr Asp Asn Asn Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
      50   55   60

TCT GGC TCC AAG TCT GGC CCC TCA GCC TCC CTG GCC ATC TCT GGG CTC   240
Ser Gly Ser Lys Ser Gly Pro Ser Ala Ser Leu Ala Ile Ser Gly Leu
65   70   75   80

CAG GCT GAG GAT GAG GCT GAT TAT TAC TGC CAG TCC TAT GAC AGC AGC   288
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
              85   90   95

CTG AAT GGT TAT GTC TTC GGA ACT GGG ACC CAG CTC ACC GTC CTA GGT   336
Leu Asn Gly Tyr Val Phe Gly Thr Gly Thr Gln Leu Thr Val Leu Gly
          100   105   110

(2) INFORMATION FOR SEQ ID NO:2:

          (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 112 amino acids
              (B) TYPE: amino acid
              (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: protein

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1   5   10   15

Lys Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Leu Gly Ala Gly
              20   25   30

Tyr Asp Val His Trp Tyr Arg Gln Leu Pro Gly Thr Ala Pro Lys Leu
          35   40   45

Leu Ile Tyr Asp Asn Asn Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
      50   55   60

Ser Gly Ser Lys Ser Gly Pro Ser Ala Ser Leu Ala Ile Ser Gly Leu
65   70   75   80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
              85   90   95

Leu Asn Gly Tyr Val Phe Gly Thr Gly Thr Gln Leu Thr Val Leu Gly
          100   105   110

(2) INFORMATION FOR SEQ ID NO:3:

          (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 357 base pairs
              (B) TYPE: nucleic acid
              (C) STRANDEDNESS: double

        (D) TOPOLOGY: unknown

    (ii) MOLECULE TYPE: cDNA

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 1..357

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG GGG   48
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1   5   10   15

TCC CTG AGA CTC TCC TGC GCA GCC TCT GGA GTC TCC CTC AGT GGA TAC   96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu Ser Gly Tyr
            20   25   30

AAG ATG AAC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG GAA TGG GTC   144
Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

TCT TCC ATT ACT GGT ATG AGT AAT TAC ATA CAC TAC TCA GAC TCA GTG   192
Ser Ser Ile Thr Gly Met Ser Asn Tyr Ile His Tyr Ser Asp Ser Val
    50   55   60

AAG GGC CGA TTC ACC ATC TCC AGA GAC AAC GCC ATG AAC TCA CTG TAT   240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Met Asn Ser Leu Tyr
65   70   75   80

CTG CAA ATG AAC AGC CTG ACA GCC GAG GAC ACG GGT GTT TAT TAT TGT   288
Leu Gln Met Asn Ser Leu Thr Ala Glu Asp Thr Gly Val Tyr Tyr Cys
                85   90   95

GCG ACA CAA CCG GGG GAG CTG GCG CCT TTT GAC CAT TGG GGC CAG GGA   336
Ala Thr Gln Pro Gly Glu Leu Ala Pro Phe Asp His Trp Gly Gln Gly
            100   105   110

ACC CTG GTC ACC GTC TCC TCA   357
Thr Leu Val Thr Val Ser Ser
        115

(2) INFORMATION FOR SEQ ID NO:4:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 119 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu Ser Gly Tyr
            20   25   30

Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

          35   40   45

Ser Ser Ile Thr Gly Met Ser Asn Tyr Ile His Tyr Ser Asp Ser Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Met Asn Ser Leu Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Thr Ala Glu Asp Thr Gly Val Tyr Tyr Cys
                85   90   95

Ala Thr Gln Pro Gly Glu Leu Ala Pro Phe Asp His Trp Gly Gln Gly
            100   105   110

Thr Leu Val Thr Val Ser Ser
        115

(2) INFORMATION FOR SEQ ID NO:5:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 119 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS:
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

        Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
        1   5   10   15

        Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu Ser Gly Tyr
                    20   25   30

        Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
                    35   40   45

        Ser Ser Ile Thr Gly Met Ser Asn Tyr Ile His Tyr Ser Asp Ser Val
            50   55   60

        Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Met Asn Ser Leu Tyr
        65   70   75   80

        Leu Gln Met Asn Ser Leu Thr Ala Glu Asp Thr Gly Val Tyr Tyr Cys
                        85   90   95

        Ala Thr Gln Pro Gly Glu Leu Ala Pro Phe Asp His Trp Gly Gln Gly
                    100   105   110

        Thr Leu Val Thr Val Ser Ser
                115

(2) INFORMATION FOR SEQ ID NO:6:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 98 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS:
            (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Glu  Val  Gln  Leu  Val  Glu  Ser  Gly  Gly  Gly  Leu  Val  Gln  Pro  Gly  Gly
1                   5                   10                  15

Ser  Leu  Arg  Leu  Ser  Cys  Ala  Ala  Ser  Gly  Phe  Thr  Phe  Ser  Ser  Tyr
          20                  25                  30

Glu  Met  Asn  Trp  Val  Arg  Gln  Ala  Pro  Gly  Lys  Gly  Leu  Glu  Trp  Val
          35                  40                  45

Ser  Tyr  Ile  Ser  Ser  Ser  Gly  Ser  Thr  Ile  Tyr  Tyr  Ala  Asp  Ser  Val
          50                  55                  60

Lys  Gly  Arg  Phe  Thr  Ile  Ser  Arg  Asp  Asn  Ala  Lys  Asn  Ser  Leu  Tyr
65                  70                  75                  80

Leu  Gln  Met  Asn  Ser  Leu  Arg  Ala  Glu  Asp  Thr  Ala  Val  Tyr  Tyr  Cys
               85                  90                  95

Ala  Arg
```


(2) INFORMATION FOR SEQ ID NO:7:

          (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 138 amino acids
               (B) TYPE: amino acid
               (C) STRANDEDNESS:
               (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met  Gly  Trp  Ser  Cys  Ile  Ile  Leu  Phe  Leu  Val  Ala  Thr  Ala  Thr  Gly
1                   5                   10                  15

Val  His  Ser  Glu  Val  Gln  Leu  Leu  Glu  Ser  Gly  Gly  Gly  Leu  Val  Gln
          20                  25                  30

Pro  Gly  Gly  Ser  Leu  Arg  Leu  Ser  Cys  Ala  Ala  Ser  Gly  Val  Ser  Leu
          35                  40                  45

Ser  Gly  Tyr  Lys  Met  Asn  Trp  Val  Arg  Gln  Ala  Pro  Gly  Lys  Gly  Leu
          50                  55                  60

Glu  Trp  Val  Ser  Ser  Ile  Thr  Gly  Met  Ser  Asn  Tyr  Ile  His  Tyr  Ser
65                  70                  75                  80

Asp  Ser  Val  Lys  Gly  Arg  Phe  Thr  Ile  Ser  Arg  Asp  Asn  Ala  Met  Asn
               85                  90                  95

Ser  Leu  Tyr  Leu  Gln  Met  Asn  Ser  Leu  Thr  Ala  Glu  Asp  Thr  Gly  Val
```

```
                100                      105                      110
    Tyr Tyr Cys Ala Thr Gln Pro Gly Glu Leu Ala Pro Phe Asp His Trp
            115                      120                  125

    Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        130                      135
```

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 138 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
    Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
    1               5                   10                  15

    Val His Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
                20                  25                  30

    Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ser Leu
            35                  40                  45

    Ser Gly Tyr Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
        50                  55                  60

    Glu Trp Val Ser Ser Ile Thr Gly Met Ser Asn Tyr Ile His Tyr Ser
    65                  70                  75                  80

    Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Met Asn
                    85                  90                  95

    Ser Leu Tyr Leu Gln Met Asn Ser Leu Thr Ala Glu Asp Thr Gly Val
            100                 105                 110

    Tyr Tyr Cys Ala Thr Gln Pro Gly Glu Leu Ala Pro Phe Asp His Trp
            115                     120                 125

    Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        130                     135
```

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 111 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: unknown

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1               5                  10              15

Lys Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Leu Gly Ala Gly
            20                  25              30

Tyr Asp Val His Trp Tyr Arg Gln Leu Pro Gly Thr Ala Pro Lys Leu
        35                  40              45

Leu Ile Tyr Asp Asn Asn Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
        50                  55              60

Ser Gly Ser Lys Ser Gly Pro Ser Ala Ser Leu Ala Ile Ser Gly Leu
65                  70                  75                  80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
                85                  90                  95

Leu Asn Gly Tyr Val Phe Gly Thr Gly Thr Gln Leu Thr Val Leu
                100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 90 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: unknown

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1               5                  10              15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
            20                  25              30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
        35                  40              45

Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
        50                  55              60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65                  70                  75                  80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 128 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1               5               10              15

Val His Ser Glu Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly
            20              25              30

Gln Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala
        35              40              45

Gly Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys
    50              55              60

Leu Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg
65              70              75              80

Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly
            85              90              95

Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser
        100             105             110

Ser Leu Asn Gly Tyr Val Phe Gly Thr Gly Thr Gln Leu Thr Val Leu
    115             120             125
```

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 130 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: unknown

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1               5               10              15

Val His Ser Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala
            20              25              30

Pro Gly Gln Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile
        35              40              45

Gly Ala Gly Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala
    50              55              60

Pro Lys Leu Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro
65              70              75              80
```

```
      Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile
                      85                  90                  95

      Thr Gly Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr
                  100                 105                 110

      Asp Ser Ser Leu Asn Gly Tyr Val Phe Gly Thr Gly Thr Gln Leu Thr
              115                 120                 125

      Val Leu
          130
```

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 6281 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: unknown

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GACGTCGCGG CCGCTCTAGG CCTCCAAAAA AGCCTCCTCA CTACTTCTGG AATAGCTCAG      60

AGGCCGAGGC GGCCTCGGCC TCTGCATAAA TAAAAAAAAT TAGTCAGCCA TGCATGGGGC     120

GGAGAATGGG CGGAACTGGG CGGAGTTAGG GGCGGGATGG GCGGAGTTAG GGGCGGGACT     180

ATGGTTGCTG ACTAATTGAG ATGCATGCTT TGCATACTTC TGCCTGCTGG GGAGCCTGGG     240

GACTTTCCAC ACCTGGTTGC TGACTAATTG AGATGCATGC TTTGCATACT TCTGCCTGCT     300

GGGGAGCCTG GGACTTTCC ACACCCTAAC TGACACACAT TCCACAGAAT TAATTCCCGG     360

GGATCGATCC GTCGACGTAC GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT     420

CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA     480

CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA     540

ATAGGGACTT TCCATTGACG TCAATGGGTG GACTATTTAC GGTAAACTGC CCACTTGGCA     600

GTACATCAAG TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG     660

CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC     720

TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT     780

GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT     840

TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG     900

ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TGGGTACGTG     960

AACCGTCAGA TCGCCTGGAG ACGCCATCGA ATTCTGAGCA CACAGGACCT CACCATGGGA    1020

TGGAGCTGTA TCATCCTCTT CTTGGTAGCA ACAGCTACAG GTGTCCACTC CGAGGTCCAA    1080
```

```
CTGCTCGAGT CTGGGGGAGG CTTGGTACAG CCTGGGGGGT CCCTGAGACT CTCCTGCGCA      1140

GCCTCTGGAG TCTCCCTCAG TGGATACAAG ATGAACTGGG TCCGCCAGGC TCCAGGGAAG      1200

GGGCTGGAAT GGGTCTCTTC CATTACTGGT ATGAGTAATT ACATACACTA CTCAGACTCA      1260

GTGAAGGGCC GATTCACCAT CTCCAGAGAC AACGCCATGA ACTCACTGTA TCTGCAAATG      1320

AACAGCCTGA CAGCCGAGGA CACGGGTGTT TATTATTGTG CGACACAACC GGGGGAGCTG      1380

GCGCCTTTTG ACCATTGGGG CCAGGGAACC CTGGTCACCG TCTCCTCAGC CTCCACCAAG      1440

GGCCCATCGG TCTTCCCCCT GGCACCCTCC TCCAAGAGCA CCTCTGGGGG CACAGCGGCC      1500

CTGGGCTGCC TGGTCAAGGA CTACTTCCCC GAACCGGTGA CGGTGTCGTG GAACTCAGGC      1560

GCCCTGACCA GCGGCGTGCA CACCTTCCCG GCTGTCCTAC AGTCCTCAGG ACTCTACTCC      1620

CTCAGCAGCG TGGTGACCGT GCCCTCCAGC AGCTTGGGCA CCCAGACCTA CATCTGCAAC      1680

GTGAATCACA AGCCCAGCAA CACCAAGGTG GACAAGAAAG TTGAGCCCAA ATCTTGTGAC      1740

AAAACTCACA CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC      1800

CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC      1860

GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC      1920

GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGG      1980

GTGGTCAGCG TCCTCACCGT CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC      2040

AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG      2100

CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGATGAGCT GACCAAGAAC      2160

CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG      2220

GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC CTCCCGTGCT GGACTCCGAC      2280

GGCTCCTTCT TCCTCTACAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC      2340

GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC      2400

TCCCTGTCTC CGGGTAAATG ATAGATATCT ACGTATGATC AGCCTCGACT GTGCCTTCTA      2460

GTTGCCAGCC ATCTGTTGTT TGCCCCTCCC CCGTGCCTTC CTTGACCCTG GAAGGTGCCA      2520

CTCCCACTGT CCTTTCCTAA TAAAATGAGG AAATTGCATC GCATTGTCTG AGTAGGTGTC      2580

ATTCTATTCT GGGGGGTGGG GTGGGGCAGG ACAGCAAGGG GGAGGATTGG GAAGACAATA      2640

GCAGGCATGC TGGGGATGCG GTGGGCTCTA TGGAACCAGC TGGGGCTCGA CAGCGCTGGA      2700

TCTCCCGATC CCCAGCTTTG CTTCTCAATT TCTTATTTGC ATAATGAGAA AAAAAGGAAA      2760

ATTAATTTTA ACACCAATTC AGTAGTTGAT TGAGCAAATG CGTTGCCAAA AAGGATGCTT      2820

TAGAGACAGT GTTCTCTGCA CAGATAAGGA CAAACATTAT TCAGAGGGAG TACCCAGAGC      2880

TGAGACTCCT AAGCCAGTGA GTGGCACAGC ATTCTAGGGA GAAATATGCT TGTCATCACC      2940

GAAGCCTGAT TCCGTAGAGC CACACCTTGG TAAGGGCCAA TCTGCTCACA CAGGATAGAG      3000
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AGGGCAGGAG CCAGGGCAGA GCATATAAGG TGAGGTAGGA TCAGTTGCTC CTCACATTTG   3060

CTTCTGACAT AGTTGTGTTG GGAGCTTGGA TAGCTTGGAC AGCTCAGGGC TGCGATTTCG   3120

CGCCAAACTT GACGGCAATC CTAGCGTGAA GGCTGGTAGG ATTTTATCCC CGCTGCCATC   3180

ATGGTTCGAC CATTGAACTG CATCGTCGCC GTGTCCCAAA ATATGGGGAT TGGCAAGAAC   3240

GGAGACCTAC CCTGGCCTCC GCTCAGGAAC GAGTTCAAGT ACTTCCAAAG AATGACCACA   3300

ACCTCTTCAG TGGAAGGTAA ACAGAATCTG GTGATTATGG GTAGGAAAAC CTGGTTCTCC   3360

ATTCCTGAGA AGAATCGACC TTTAAAGGAC AGAATTAATA TAGTTCTCAG TAGAGAACTC   3420

AAAGAACCAC CACGAGGAGC TCATTTTCTT GCCAAAAGTT TGGATGATGC CTTAAGACTT   3480

ATTGAACAAC CGGAATTGGC AAGTAAAGTA GACATGGTTT GGATAGTCGG AGGCAGTTCT   3540

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CAGGAATTTG AAAGTGACAC GTTTTTCCCA GAAATTGATT TGGGGAAATA TAAACTTCTC   3660

CCAGAATACC CAGGCGTCCT CTCTGAGGTC CAGGAGGAAA AAGGCATCAA GTATAAGTTT   3720

GAAGTCTACG AGAAGAAAGA CTAACAGGAA GATGCTTTCA AGTTCTCTGC TCCCCTCCTA   3780

AAGCTATGCA TTTTTATAAG ACCATGGGAC TTTTGCTGGC TTTAGATCAG CCTCGACTGT   3840

GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCTTCCT TGACCCTGGA   3900

AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA ATTGCATCGC ATTGTCTGAG   3960

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GAGTGTATGA CTGCGGCCGC GATCCCGTCG AGAGCTTGGC GTAATCATGG TCATAGCTGT   4140

TTCCTGTGTG AAATTGTTAT CCGCTCACAA TTCCACACAA CATACGAGCC GGAAGCATAA   4200

AGTGTAAAGC CTGGGGTGCC TAATGAGTGA GCTAACTCAC ATTAATTGCG TTGCGCTCAC   4260

TGCCCGCTTT CCAGTCGGGA AACCTGTCGT GCCAGCTGCA TTAATGAATC GGCCAACGCG   4320

CGGGGAGAGG CGGTTTGCGT ATTGGGCGCT CTTCCGCTTC CTCGCTCACT GACTCGCTGC   4380

GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA ATACGGTTAT   4440

CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC AAAAGGCCAG CAAAAGGCCA   4500

GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC CCTGACGAGC   4560

ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC GACAGGACTA TAAAGATACC   4620

AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT TCCGACCCTG CCGCTTACCG   4680

GATACCTGTC CGCCTTTCTC CCTTCGGGAA GCGTGGCGCT TTCTCAATGC TCACGCTGTA   4740

GGTATCTCAG TTCGGTGTAG GTCGTTCGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG   4800

TTCAGCCCGA CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAC CCGGTAAGAC   4860
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ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT TAGCAGAGCG AGGTATGTAG    4920

GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC CTAACTACGG CTACACTAGA AGGACAGTAT    4980

TTGGTATCTG CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT    5040

CCGGCAAACA AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC    5100

GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT GACGCTCAGT    5160

GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT ATCAAAAAGG ATCTTCACCT    5220

AGATCCTTTT AAATTAAAAA TGAAGTTTTA AATCAATCTA AGTATATAT GAGTAAACTT     5280

GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC TGTCTATTTC    5340

GTTCATCCAT AGTTGCCTGA CTCCCCGTCG TGTAGATAAC TACGATACGG GAGGGCTTAC    5400

CATCTGGCCC CAGTGCTGCA ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT    5460

CAGCAATAAA CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG    5520

CCTCCATCCA GTCTATTAAT TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG CCAGTTAATA    5580

GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT GTCACGCTCG TCGTTTGGTA    5640

TGGCTTCATT CAGCTCCGGT TCCCAACGAT CAAGGCGAGT TACATGATCC CCCATGTTGT    5700

GCAAAAAAGC GGTTAGCTCC TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGCAG    5760

TGTTATCACT CATGGTTATG GCAGCACTGC ATAATTCTCT TACTGTCATG CCATCCGTAA    5820

GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG TGTATGCGGC    5880

GACCGAGTTG CTCTTGCCCG GCGTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT    5940

TAAAAGTGCT CATCATTGGA AAACGTTCTT CGGGGCGAAA ACTCTCAAGG ATCTTACCGC    6000

TGTTGAGATC CAGTTCGATG TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA    6060

CTTTCACCAG CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA    6120

TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT TATTGAAGCA    6180

TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA ATGTATTTAG AAAAATAAAC    6240

AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC T                        6281
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(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 5679 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: unknown

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

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GACGTCGCGG CCGCTCTAGG CCTCCAAAAA AGCCTCCTCA CTACTTCTGG AATAGCTCAG      60

AGGCCGAGGC GGCCTCGGCC TCTGCATAAA TAAAAAAAAT TAGTCAGCCA TGCATGGGGC     120

GGAGAATGGG CGGAACTGGG CGGAGTTAGG GGCGGGATGG GCGGAGTTAG GGGCGGGACT     180

ATGGTTGCTG ACTAATTGAG ATGCATGCTT TGCATACTTC TGCCTGCTGG GGAGCCTGGG     240

GACTTTCCAC ACCTGGTTGC TGACTAATTG AGATGCATGC TTTGCATACT TCTGCCTGCT     300

GGGGAGCCTG GGGACTTTCC ACACCCTAAC TGACACACAT TCCACAGAAT TAATTCCCGG     360

GGATCGATCC GTCGACGTAC GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT     420

CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA     480

CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA     540

ATAGGGACTT TCCATTGACG TCAATGGGTG GACTATTTAC GGTAAACTGC CCACTTGGCA     600

GTACATCAAG TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG     660

CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC     720

TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT     780

GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT     840

TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG     900

ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TGGGTACGTG     960

AACCGTCAGA TCGCCTGGAG ACGCCATCGA ATTCTGAGCA CACAGGACCT CACCATGGGA    1020

TGGAGCTGTA TCATCCTCTT CTTGGTAGCA ACAGCTACAG GTGTCCACTC CGAGCTCACG    1080

CAGCCGCCCT CAGTCTCTGC GGCCCCAGGA CAGAAGGTCA CCATCTCCTG CACTGGGAGC    1140

AGCTCCAACC TCGGGGCAGG TTATGATGTT CACTGGTACC GGCAACTTCC AGGGACAGCC    1200

CCCAAACTCC TCATCTATGA TAACAACAAT CGGCCCTCAG GGGTCCCTGA CCGATTCTCT    1260

GGCTCCAAGT CTGGCCCCTC AGCCTCCCTG GCCATCTCTG GCTCCAGGC TGAGGATGAG    1320

GCTGATTATT ACTGCCAGTC CTATGACAGC AGCCTGAATG GTTATGTCTT CGGAACTGGG    1380

ACCCAGCTCA CCGTCCTAGG TCAGCCCAAG CTGCCCCCT CGGTCACTCT GTTCCCGCCC    1440

TCCTCTGAGG AGCTTCAAGC CAACAAGGCC ACACTGGTGT GTCTCATAAG TGACTTCTAC    1500

CCGGGAGCCG TGACAGTGGC CTGGAAGGCA ATTAGCAGCC CCGTCAAGGC GGGAGTGGAG    1560

ACCACCACAC CCTCCAAACA AAGCAACAAC AAGTACGCGG CCAGCAGCTA TCTGAGCCTG    1620

ACGCCTGAGC AGTGGAAGTC CCACAGAAGG TACAGCTGCC AGGTCACGCA TGAAGGGAGC    1680

ACCGTGGAGA AGACAGTGGC CCCTACAGAA TGTTCATAGT TCTAGATCTA CGTATGATCA    1740

GCCTCGACTG TGCCTTCTAG TTGCCAGCCA TCTGTTGTTT GCCCCTCCCC CGTGCCTTCC    1800

TTGACCCTGG AAGGTGCCAC TCCCACTGTC CTTTCCTAAT AAAATGAGGA AATTGCATCG    1860

CATTGTCTGA GTAGGTGTCA TTCTATTCTG GGGGGTGGGG TGGGGCAGGA CAGCAAGGGG    1920
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GAGGATTGGG AAGACAATAG CAGGCATGCT GGGGATGCGG TGGGCTCTAT GGAACCAGCT      1980

GGGGCTCGAC AGCTCGAGCT AGCTTTGCTT CTCAATTTCT TATTTGCATA ATGAGAAAAA      2040

AAGGAAAATT AATTTTAACA CCAATTCAGT AGTTGATTGA GCAAATGCGT TGCCAAAAAG      2100

GATGCTTTAG AGACAGTGTT CTCTGCACAG ATAAGGACAA ACATTATTCA GAGGGAGTAC      2160

CCAGAGCTGA GACTCCTAAG CCAGTGAGTG GCACAGCATT CTAGGGAGAA ATATGCTTGT      2220

CATCACCGAA GCCTGATTCC GTAGAGCCAC ACCTTGGTAA GGGCCAATCT GCTCACACAG      2280

GATAGAGAGG GCAGGAGCCA GGGCAGAGCA TATAAGGTGA GGTAGGATCA GTTGCTCCTC      2340

ACATTTGCTT CTGACATAGT TGTGTTGGGA GCTTGGATCG ATCCACCATG GTTGAACAAG      2400

ATGGATTGCA CGCAGGTTCT CCGGCCGCTT GGGTGGAGAG GCTATTCGGC TATGACTGGG      2460

CACAACAGAC AATCGGCTGC TCTGATGCCG CCGTGTTCCG GCTGTCAGCG CAGGGGCGCC      2520

CGGTTCTTTT TGTCAAGACC GACCTGTCCG GTGCCCTGAA TGAACTGCAG GACGAGGCAG      2580

CGCGGCTATC GTGGCTGGCC ACGACGGGCG TTCCTTGCGC AGCTGTGCTC GACGTTGTCA      2640

CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC GGGGCAGGAT CTCCTGTCAT      2700

CTCACCTTGC TCCTGCCGAG AAAGTATCCA TCATGGCTGA TGCAATGCGG CGGCTGCATA      2760

CGCTTGATCC GGCTACCTGC CCATTCGACC ACCAAGCGAA ACATCGCATC GAGCGAGCAC      2820

GTACTCGGAT GGAAGCCGGT CTTGTCGATC AGGATGATCT GGACGAAGAG CATCAGGGGC      2880

TCGCGCCAGC CGAACTGTTC GCCAGGCTCA AGGCGCGCAT GCCCGACGGC GAGGATCTCG      2940

TCGTGACCCA TGGCGATGCC TGCTTGCCGA ATATCATGGT GGAAAATGGC CGCTTTTCTG      3000

GATTCATCGA CTGTGGCCGG CTGGGTGTGG CGGACCGCTA TCAGGACATA GCGTTGGCTA      3060

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GTATCGCCGC TCCCGATTCG CAGCGCATCG CCTTCTATCG CCTTCTTGAC GAGTTCTTCT      3180

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CGGCTGGATG ATCCTCCAGC GCGGGGATCT CATGCTGGAG TTCTTCGCCC ACCCCAACTT      3360

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AGCATTTTTT TCACTGCATT CTAGTTGTGG TTTGTCCAAA CTCATCAATG TATCTTATCA      3480

TGTCTGGATC GCGGCCGCGA TCCCGTCGAG AGCTTGGCGT AATCATGGTC ATAGCTGTTT      3540

CCTGTGTGAA ATTGTTATCC GCTCACAATT CCACACAACA TACGAGCCGG AAGCATAAAG      3600

TGTAAAGCCT GGGGTGCCTA ATGAGTGAGC TAACTCACAT TAATTGCGTT GCGCTCACTG      3660

CCCGCTTTCC AGTCGGGAAA CCTGTCGTGC CAGCTGCATT AATGAATCGG CCAACGCGCG      3720

GGGAGAGGCG GTTTGCGTAT TGGGCGCTCT TCCGCTTCCT CGCTCACTGA CTCGCTGCGC      3780
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TCGGTCGTTC GGCTGCGGCG AGCGGTATCA GCTCACTCAA AGGCGGTAAT ACGGTTATCC   3840

ACAGAATCAG GGGATAACGC AGGAAAGAAC ATGTGAGCAA AAGGCCAGCA AAAGGCCAGG   3900

AACCGTAAAA AGGCCGCGTT GCTGGCGTTT TTCCATAGGC TCCGCCCCCC TGACGAGCAT   3960

CACAAAAATC GACGCTCAAG TCAGAGGTGG CGAAACCCGA CAGGACTATA AAGATACCAG   4020

GCGTTTCCCC CTGGAAGCTC CCTCGTGCGC TCTCCTGTTC CGACCCTGCC GCTTACCGGA   4080

TACCTGTCCG CCTTTCTCCC TTCGGGAAGC GTGGCGCTTT CTCAATGCTC ACGCTGTAGG   4140

TATCTCAGTT CGGTGTAGGT CGTTCGCTCC AAGCTGGGCT GTGTGCACGA ACCCCCCGTT   4200

CAGCCCGACC GCTGCGCCTT ATCCGGTAAC TATCGTCTTG AGTCCAACCC GGTAAGACAC   4260

GACTTATCGC CACTGGCAGC AGCCACTGGT AACAGGATTA GCAGAGCGAG GTATGTAGGC   4320

GGTGCTACAG AGTTCTTGAA GTGGTGGCCT AACTACGGCT ACACTAGAAG GACAGTATTT   4380

GGTATCTGCG CTCTGCTGAA GCCAGTTACC TTCGGAAAAA GAGTTGGTAG CTCTTGATCC   4440

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TCTGACAGTT ACCAATGCTT AATCAGTGAG GCACCTATCT CAGCGATCTG TCTATTTCGT   4740

TCATCCATAG TTGCCTGACT CCCCGTCGTG TAGATAACTA CGATACGGGA GGGCTTACCA   4800

TCTGGCCCCA GTGCTGCAAT GATACCGCGA GACCCACGCT CACCGGCTCC AGATTTATCA   4860

GCAATAAACC AGCCAGCCGG AAGGGCCGAG CGCAGAAGTG GTCCTGCAAC TTTATCCGCC   4920

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GCTTCATTCA GCTCCGGTTC CCAACGATCA AGGCGAGTTA CATGATCCCC CATGTTGTGC   5100

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CCGAGTTGCT CTTGCCCGGC GTCAATACGG GATAATACCG CGCCACATAG CAGAACTTTA   5340

AAAGTGCTCA TCATTGGAAA ACGTTCTTCG GGGCGAAAAC TCTCAAGGAT CTTACCGCTG   5400

TTGAGATCCA GTTCGATGTA ACCCACTCGT GCACCCAACT GATCTTCAGC ATCTTTTACT   5460

TTCACCAGCG TTTCTGGGTG AGCAAAAACA GGAAGGCAAA ATGCCGCAAA AAAGGGAATA   5520

AGGGCGACAC GGAAATGTTG AATACTCATA CTCTTCCTTT TTCAATATTA TTGAAGCATT   5580

TATCAGGGTT ATTGTCTCAT GAGCGGATAC ATATTTGAAT GTATTTAGAA AAATAAACAA   5640

ATAGGGGTTC CGCGCACATT TCCCCGAAAA GTGCCACCT              5679
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(2) INFORMATION FOR SEQ ID NO:15:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1442 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: unknown

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

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GAATTCTGAG CACACAGGAC CTCACCATGG GATGGAGCTG TATCATCCTC TTCTTGGTAG    60

CAACAGCTAC AGGTGTCCAC TCCGAGGTGC AGCTGGTGGA GTCTGGGGGA GGCTTGGTAC   120

AGCCTGGGGG GTCCCTGAGA CTCTCCTGCG CAGCCTCTGG AGTCTCCCTC AGTGGATACA   180

AGATGAACTG GGTCCGCCAG GCTCCAGGGA AGGGGCTGGA ATGGGTCTCT TCCATTACTG   240

GTATGAGTAA TTACATACAC TACTCAGACT CAGTGAAGGG CCGATTCACC ATCTCCAGAG   300

ACAACGCCAT GAACTCACTG TATCTGCAAA TGAACAGCCT GACAGCCGAG GACACGGGTG   360

TTTATTATTG TGCGACACAA CCGGGGGAGC TGGCGCCTTT TGACCATTGG GGCCAGGGAA   420

CCCTGGTCAC CGTCTCCTCA GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCT   480

CCTCCAAGAG CACCTCTGGG GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC   540

CCGAACCGGT GACGGTGTCG TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC   600

CGGCTGTCCT ACAGTCCTCA GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA   660

GCAGCTTGGG CACCCAGACC TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG   720

TGGACAAGAA AGTTGAGCCC AAATCTTGTG ACAAAACTCA CACATGCCCA CCGTGCCCAG   780

CACCTGAACT CCTGGGGGGA CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC   840

TCATGATCTC CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC   900

CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC   960

CGCGGGAGGA GCAGTACAAC AGCACGTACC GGGTGGTCAG CGTCCTCACC GTCCTGCACC  1020

AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCAGCCC  1080

CCATCGAGAA AACCATCTCC AAAGCCAAAG GCAGCCCCG AGAACCACAG GTGTACACCC   1140

TGCCCCCATC CCGGGATGAG CTGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG  1200

GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT  1260

ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA  1320

CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG  1380

CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA TGATAGATAT  1440
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CT   1442

(2) INFORMATION FOR SEQ ID NO:16:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 762 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: unknown

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

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GAATTCTGAG CACACAGGAC CTCACCATGG GATGGAGCTG TATCATCCTC TTCTTGGTAG        60

CAACAGCTAC AGGTGTCCAC TCCCAGTCTG TGTTGACGCA GCCGCCCTCA GTCTCTGCGG       120

CCCCAGGACA GAAGGTCACC ATCTCCTGCA CTGGGAGCAG CTCCAACCTC GGGGCAGGTT       180

ATGATGTTCA CTGGTACCGG CAACTTCCAG GGACAGCCCC CAAACTCCTC ATCTATGATA       240

ACAACAATCG GCCCTCAGGG GTCCCTGACC GATTCTCTGG CTCCAAGTCT GGCCCCTCAG       300

CCTCCCTGGC CATCTCTGGG CTCCAGGCTG AGGATGAGGC TGATTATTAC TGCCAGTCCT       360

ATGACAGCAG CCTGAATGGT TATGTCTTCG GAACTGGGAC CCAGCTCACC GTCCTAGGTC       420

AGCCCAAGGC TGCCCCCTCG GTCACTCTGT TCCCGCCCTC CTCTGAGGAG CTTCAAGCCA       480

ACAAGGCCAC ACTGGTGTGT CTCATAAGTG ACTTCTACCC GGGAGCCGTG ACAGTGGCCT       540

GGAAGGCAAT TAGCAGCCCC GTCAAGGCGG GAGTGGAGAC CACCACACCC TCCAAACAAA       600

GCAACAACAA GTACGCGGCC AGCAGCTATC TGAGCCTGAC GCCTGAGCAG TGGAAGTCCC       660

ACAGAAGGTA CAGCTGCCAG GTCACGCATG AAGGGAGCAC CGTGGAGAAG ACAGTGGCCC       720

CTACAGAATG TTCATAGTTC TAGATCTACG TATGATCAGC CT                          762
```

(2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 6 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

    Glu Val Gln Leu Leu Glu
    1   5

(2) INFORMATION FOR SEQ ID NO:18:

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        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 6 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS:
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: peptide




       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

        Glu Val Gln Leu Val Glu
        1               5

(2) INFORMATION FOR SEQ ID NO:19:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 1899 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: unknown

       (ii) MOLECULE TYPE: cDNA


       (ix) FEATURE:
            (A) NAME/KEY: CDS
            (B) LOCATION: 14..1735


       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
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GGGGCAAATA ACA ATG GAG TTG CTA ATC CTC AAA GCA AAT GCA ATT ACC          49
           Met Glu Leu Leu Ile Leu Lys Ala Asn Ala Ile Thr
            1               5                   10

ACA ATC CTC ACT GCA GTC ACA TTT TGT TTT GCT TCT GGT CAA AAC ATC          97
Thr Ile Leu Thr Ala Val Thr Phe Cys Phe Ala Ser Gly Gln Asn Ile
        15                  20                  25

ACT GAA GAA TTT TAT CAA TCA ACA TGC AGT GCA GTT AGC AAA GGC TAT         145
Thr Glu Glu Phe Tyr Gln Ser Thr Cys Ser Ala Val Ser Lys Gly Tyr
    30                  35                  40

CTT AGT GCT CTG AGA ACT GGT TGG TAT ACC AGT GTT ATA ACT ATA GAA         193
Leu Ser Ala Leu Arg Thr Gly Trp Tyr Thr Ser Val Ile Thr Ile Glu
45                  50                  55                  60

TTA AGT AAT ATC AAG GAA AAT AAG TGT AAT GGA ACA GAT GCT AAG GTA         241
Leu Ser Asn Ile Lys Glu Asn Lys Cys Asn Gly Thr Asp Ala Lys Val
                65                  70                  75

AAA TTG ATA AAA CAA GAA TTA GAT AAA TAT AAA AAT GCT GTA ACA GAA         289
Lys Leu Ile Lys Gln Glu Leu Asp Lys Tyr Lys Asn Ala Val Thr Glu
                80                  85                  90

TTG CAG TTG CTC ATG CAA AGC ACA CCA CCA ACA AAC AAT CGA GCC AGA         337
Leu Gln Leu Leu Met Gln Ser Thr Pro Pro Thr Asn Asn Arg Ala Arg
                95                  100                 105
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AGA GAA CTA CCA AGG TTT ATG AAT TAT ACA CTC AAC AAT GCC AAA AAA        385
Arg Glu Leu Pro Arg Phe Met Asn Tyr Thr Leu Asn Asn Ala Lys Lys
    110             115             120

ACC AAT GTA ACA TTA AGC AAG AAA AGG AAA AGA AGA TTT CTT GGT TTT        433
Thr Asn Val Thr Leu Ser Lys Lys Arg Lys Arg Arg Phe Leu Gly Phe
125             130             135             140

TTG TTA GGT GTT GGA TCT GCA ATC GCC AGT GGC GTT GCT GTA TCT AAG        481
Leu Leu Gly Val Gly Ser Ala Ile Ala Ser Gly Val Ala Val Ser Lys
                145             150             155

GTC CTG CAC CTA GAA GGG GAA GTG AAC AAG ATC AAA AGT GCT CTA CTA        529
Val Leu His Leu Glu Gly Glu Val Asn Lys Ile Lys Ser Ala Leu Leu
            160             165             170

TCC ACA AAC AAG GCT GTA GTC AGC TTA TCA AAT GGA GTT AGT GTC TTA        577
Ser Thr Asn Lys Ala Val Val Ser Leu Ser Asn Gly Val Ser Val Leu
            175             180             185

ACC AGC AAA GTG TTA GAC CTC AAA AAC TAT ATA GAT AAA CAA TTG TTA        625
Thr Ser Lys Val Leu Asp Leu Lys Asn Tyr Ile Asp Lys Gln Leu Leu
    190             195             200

CCT ATT GTG AAC AAG CAA AGC TGC AGC ATA TCA AAT ATA GAA ACT GTG        673
Pro Ile Val Asn Lys Gln Ser Cys Ser Ile Ser Asn Ile Glu Thr Val
205             210             215             220

ATA GAG TTC CAA CAA AAG AAC AAC AGA CTA CTA GAG ATT ACC AGG GAA        721
Ile Glu Phe Gln Gln Lys Asn Asn Arg Leu Leu Glu Ile Thr Arg Glu
                225             230             235

TTT AGT GTT AAT GCA GGT GTA ACT ACA CCT GTA AGC ACT TAC ATG TTA        769
Phe Ser Val Asn Ala Gly Val Thr Thr Pro Val Ser Thr Tyr Met Leu
            240             245             250

ACT AAT AGT GAA TTA TTG TCA TTA ATC AAT GAT ATG CCT ATA ACA AAT        817
Thr Asn Ser Glu Leu Leu Ser Leu Ile Asn Asp Met Pro Ile Thr Asn
            255             260             265

GAT CAG AAA AAG TTA ATG TCC AAC AAT GTT CAA ATA GTT AGA CAG CAA        865
Asp Gln Lys Lys Leu Met Ser Asn Asn Val Gln Ile Val Arg Gln Gln
    270             275             280

AGT TAC TCT ATC ATG TCC ATA ATA AAA GAG GAA GTC TTA GCA TAT GTA        913
Ser Tyr Ser Ile Met Ser Ile Ile Lys Glu Glu Val Leu Ala Tyr Val
285             290             295             300

GTA CAA TTA CCA CTA TAT GGT GTT ATA GAT ACA CCC TGT TGG AAA CTA        961
Val Gln Leu Pro Leu Tyr Gly Val Ile Asp Thr Pro Cys Trp Lys Leu
                305             310             315

CAC ACA TCC CCT CTA TGT ACA ACC AAC ACA AAA GAA GGG TCC AAC ATC       1009
His Thr Ser Pro Leu Cys Thr Thr Asn Thr Lys Glu Gly Ser Asn Ile
            320             325             330

TGT TTA ACA AGA ACT GAC AGA GGA TGG TAC TGT GAC AAT GCA GGA TCA       1057
Cys Leu Thr Arg Thr Asp Arg Gly Trp Tyr Cys Asp Asn Ala Gly Ser
        335             340             345

GTA TCT TTC TTC CCA CAA GCT GAA ACA TGT AAA GTT CAA TCA AAT CGA       1105
Val Ser Phe Phe Pro Gln Ala Glu Thr Cys Lys Val Gln Ser Asn Arg
    350             355             360
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GTA TTT TGT GAC ACA ATG AAC AGT TTA ACA TTA CCA AGT GAA ATA AAT      1153
Val Phe Cys Asp Thr Met Asn Ser Leu Thr Leu Pro Ser Glu Ile Asn
365             370             375             380

CTC TGC AAT GTT GAC ATA TTC AAC CCC AAA TAT GAT TGT AAA ATT ATG      1201
Leu Cys Asn Val Asp Ile Phe Asn Pro Lys Tyr Asp Cys Lys Ile Met
            385             390             395

ACT TCA AAA ACA GAT GTA AGC AGC TCC GTT ATC ACA TCT CTA GGA GCC      1249
Thr Ser Lys Thr Asp Val Ser Ser Ser Val Ile Thr Ser Leu Gly Ala
        400             405             410

ATT GTG TCA TGC TAT GGC AAA ACT AAA TGT ACA GCA TCC AAT AAA AAT      1297
Ile Val Ser Cys Tyr Gly Lys Thr Lys Cys Thr Ala Ser Asn Lys Asn
        415             420             425

CGT GGA ATC ATA AAG ACA TTT TCT AAC GGG TGC GAT TAT GTA TCA AAT      1345
Arg Gly Ile Ile Lys Thr Phe Ser Asn Gly Cys Asp Tyr Val Ser Asn
    430             435             440

AAA GGG ATG GAC ACT GTG TCT GTA GGT AAC ACA TTA TAT TAT GTA AAT      1393
Lys Gly Met Asp Thr Val Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn
445             450             455             460

AAG CAA GAA GGT AAA AGT CTC TAT GTA AAA GGT GAA CCA ATA ATA AAT      1441
Lys Gln Glu Gly Lys Ser Leu Tyr Val Lys Gly Glu Pro Ile Ile Asn
            465             470             475

TTC TAT GAC CCA TTA GTA TTC CCC TCT GAT GAA TTT GAT GCA TCA ATA      1489
Phe Tyr Asp Pro Leu Val Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile
            480             485             490

TCT CAA GTC AAC GAG AAG ATT AAC CAG AGC CTA GCA TTT ATT CGT AAA      1537
Ser Gln Val Asn Glu Lys Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys
            495             500             505

TCC GAT GAA TTA TTA CAT AAT GTA AAT GCT GGT AAA TCC ACC ACA AAT      1585
Ser Asp Glu Leu Leu His Asn Val Asn Ala Gly Lys Ser Thr Thr Asn
    510             515             520

ATC ATG ATA ACT ACT ATA ATT ATA GTG ATT ATA GTA ATA TTG TTA TCA      1633
Ile Met Ile Thr Thr Ile Ile Ile Val Ile Ile Val Ile Leu Leu Ser
525             530             535             540

TTA ATT GCT GTT GGA CTG CTC TTA TAC TGT AAG GCC AGA AGC ACA CCA      1681
Leu Ile Ala Val Gly Leu Leu Leu Tyr Cys Lys Ala Arg Ser Thr Pro
            545             550             555

GTC ACA CTA AGC AAA GAT CAA CTG AGT GGT ATA AAT AAT ATT GCA TTT      1729
Val Thr Leu Ser Lys Asp Gln Leu Ser Gly Ile Asn Asn Ile Ala Phe
            560             565             570

AGT AAC TAAATAAAAA TAGCACCTAA TCATGTTCTT ACAATGGTTT ACTATCTGCT      1785
Ser Asn


CATAGACAAC CCATCTGTCA TTGGATTTTC TTAAAATCTG AACTTCATCG AAACTCTCAT      1845

CTATAAACCA TCTCACTTAC ACTATTTAAG TAGATTCCTA GTTTATAGTT ATAT      1899
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(2) INFORMATION FOR SEQ ID NO:20:

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            (i)  SEQUENCE CHARACTERISTICS:
                 (A) LENGTH: 574 amino acids
                 (B) TYPE: amino acid
                 (D) TOPOLOGY: linear

           (ii)  MOLECULE TYPE: protein

           (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Glu Leu Leu Ile Leu Lys Ala Asn Ala Ile Thr Thr Ile Leu Thr
 1               5                  10                  15

Ala Val Thr Phe Cys Phe Ala Ser Gly Gln Asn Ile Thr Glu Glu Phe
                20                  25                  30

Tyr Gln Ser Thr Cys Ser Ala Val Ser Lys Gly Tyr Leu Ser Ala Leu
            35                  40                  45

Arg Thr Gly Trp Tyr Thr Ser Val Ile Thr Ile Glu Leu Ser Asn Ile
        50                  55                  60

Lys Glu Asn Lys Cys Asn Gly Thr Asp Ala Lys Val Lys Leu Ile Lys
65                  70                  75                  80

Gln Glu Leu Asp Lys Tyr Lys Asn Ala Val Thr Glu Leu Gln Leu Leu
                85                  90                  95

Met Gln Ser Thr Pro Pro Thr Asn Asn Arg Ala Arg Arg Glu Leu Pro
            100                 105                 110

Arg Phe Met Asn Tyr Thr Leu Asn Asn Ala Lys Lys Thr Asn Val Thr
        115                 120                 125

Leu Ser Lys Lys Arg Lys Arg Arg Phe Leu Gly Phe Leu Leu Gly Val
    130                 135                 140

Gly Ser Ala Ile Ala Ser Gly Val Ala Val Ser Lys Val Leu His Leu
145                 150                 155                 160

Glu Gly Glu Val Asn Lys Ile Lys Ser Ala Leu Leu Ser Thr Asn Lys
                165                 170                 175

Ala Val Val Ser Leu Ser Asn Gly Val Ser Val Leu Thr Ser Lys Val
            180                 185                 190

Leu Asp Leu Lys Asn Tyr Ile Asp Lys Gln Leu Leu Pro Ile Val Asn
        195                 200                 205

Lys Gln Ser Cys Ser Ile Ser Asn Ile Glu Thr Val Ile Glu Phe Gln
    210                 215                 220

Gln Lys Asn Asn Arg Leu Leu Glu Ile Thr Arg Glu Phe Ser Val Asn
225                 230                 235                 240

Ala Gly Val Thr Thr Pro Val Ser Thr Tyr Met Leu Thr Asn Ser Glu
            245                 250                 255

Leu Leu Ser Leu Ile Asn Asp Met Pro Ile Thr Asn Asp Gln Lys Lys
        260                 265                 270

Leu Met Ser Asn Asn Val Gln Ile Val Arg Gln Gln Ser Tyr Ser Ile
    275                 280                 285
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Met Ser Ile Ile Lys Glu Glu Val Leu Ala Tyr Val Val Gln Leu Pro
    290             295             300

Leu Tyr Gly Val Ile Asp Thr Pro Cys Trp Lys Leu His Thr Ser Pro
305             310             315             320

Leu Cys Thr Thr Asn Thr Lys Glu Gly Ser Asn Ile Cys Leu Thr Arg
            325             330             335

Thr Asp Arg Gly Trp Tyr Cys Asp Asn Ala Gly Ser Val Ser Phe Phe
            340             345             350

Pro Gln Ala Glu Thr Cys Lys Val Gln Ser Asn Arg Val Phe Cys Asp
        355             360             365

Thr Met Asn Ser Leu Thr Leu Pro Ser Glu Ile Asn Leu Cys Asn Val
    370             375             380

Asp Ile Phe Asn Pro Lys Tyr Asp Cys Lys Ile Met Thr Ser Lys Thr
385             390             395             400

Asp Val Ser Ser Ser Val Ile Thr Ser Leu Gly Ala Ile Val Ser Cys
            405             410             415

Tyr Gly Lys Thr Lys Cys Thr Ala Ser Asn Lys Asn Arg Gly Ile Ile
        420             425             430

Lys Thr Phe Ser Asn Gly Cys Asp Tyr Val Ser Asn Lys Gly Met Asp
    435             440             445

Thr Val Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn Lys Gln Glu Gly
    450             455             460

Lys Ser Leu Tyr Val Lys Gly Glu Pro Ile Ile Asn Phe Tyr Asp Pro
465             470             475             480

Leu Val Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile Ser Gln Val Asn
            485             490             495

Glu Lys Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys Ser Asp Glu Leu
        500             505             510

Leu His Asn Val Asn Ala Gly Lys Ser Thr Thr Asn Ile Met Ile Thr
    515             520             525

Thr Ile Ile Ile Val Ile Ile Val Ile Leu Leu Ser Leu Ile Ala Val
    530             535             540

Gly Leu Leu Leu Tyr Cys Lys Ala Arg Ser Thr Pro Val Thr Leu Ser
545             550             555             560

Lys Asp Gln Leu Ser Gly Ile Asn Asn Ile Ala Phe Ser Asn
            565             570
```

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1   5   10   15

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/395, 39/42; C12Q 1/00, 1/70; G01N 33/53
US CL : 424/130.1, 141.1, 147.1; 435/4, 5, 7.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 141.1, 147.1; 435/4, 5, 7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,81(,524 A (BRAMS et al) 22 September 1998, cols. 12- 20.	1, 4, 10-15
Y		2, 3
X	US 5,824,307 A (JOHNSON) 20 October 1998, cols. 4-6.	1, 4, 10-15
Y		2, 3
X	US 5,880,104 A (LI et al) 09 March 1999, cols. 6-10.	1, 4, 10-15
Y		2, 3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 AUGUST 2000	05 SEP 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer BRETT NELSON
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)★

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, DIALOG, MEDLINE
search terms: RSV, respiratory syncytial, monoclonal, antibodies, human, humanized, F protein, diagnostics, passive
immunization, therapy, treatment